Serum Placental Growth Factor levels in pre-eclampsia and its association with fetal outcomes: A case control study in Ghana

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Abstract

Introduction: Placental growth factor (PlGF) deficiency has been implicated in the pathophysiology of pre-eclampsia resulting in maternal endothelial cell dysfunction and fetal complications. This study determined the association between maternal serum levels of PlGF in pre-eclampsia and prerinatal outcomes in Ghanaian women.

Methods: This case-control study comprising of 60 pre-eclamptic (cases) and 60 normotensive pregnant women with no complications (controls) was carried out from September 2018 to February 2019. Participants' blood samples were taken at presentation, during active labour and 24 hours after delivery. The PlGF ELISA kit was used to measure the maternal serum level of the angiogenic factor. Some maternal characteristics were correlated with fetal characteristics to establish associations. Statistical significance was set at a probability of 0.05.

Results: The median serum level of PlGF in cases was significantly lower than controls (78.7pg/ml versus 1016.7pg/ml respectively, ($p<0.001$)). Likewise, the median level of PlGF in cases (65.1pg/ml) during delivery was lower than in controls (202.6pg/ml) ($p<0.001$). The low serum level of PlGF was associated with low birth weight (R2=0.2541). Pre-eclampsia was associated with low birth weight 3.3 ± 0.5kg verses 2.3 ± 0.9 kg in control and pre-eclamptic babies respectively.

Conclusion: A low PlGF level in pre-eclamptic women is associated with adverse pregnancy outcome. Therefore, perinatal outcomes may improve if there is regular clinical monitoring of PlGF levels for women with pre-eclampsia.

Keywords: Ghanaian women, neonatal outcome, placenta growth factor, Pre-eclampsia.

Introduction

Hypertensive disorders of pregnancy (HDP) are a principal cause of maternal and neonatal morbidity and mortality globally; however, their burden is higher in low- middle-income countries (LMICs).[1,2] Pre-eclampsia is the prototype of HDP developed after 20 weeks of gestation and charaterized by an increase in systolic blood pressure of 140mmHg or diastolic pressure of 90mmHg or more associated with proteinuria of more than 300mg/L in urine/24h.[3,4] It remains a major clinical challenge and affects about 2-8% of pregnant women worldwide.[5] Some studies have

attributed a genetic component to its aetiology and its said to have an estimated heredity of about 55%.[6] It remains a disease of important public health concern because of the high fatality rate. Whereas other causes of maternal mortalities can have interventions in place to prevent them, there is no effective intervention to prevent pre-eclampsia and the only known cure is delivery of the baby and placenta.[7]

In the United States of America, 4% of pregnancies are affected by pre-eclampsia and it is the second leading cause of maternal mortality.[8] In Ghana, it is one of the leading causes of maternal and neonatal mortality. A study done in 2013 in one of Ghana's tertiary hospitals reported the prevalence of pre-eclampsia at 7.9% and constituted 38% of HDPs.[9] If left untreated, pre-eclampsia can lead to eclampsia. Maternal complications include renal failure, hemolysis, elevated liver enzymes, and thrombocytopenia (HELLP syndrome), liver failure, cerebral edema with seizures and ultimately death and these complications can be life threatening.[10,11] The fetus is also not spared of the many complications of pre-eclampsia such as prematurity, intrauterine growth restriction, low APGAR scores, still birth and low birth weight.

In spite of the major progress made during the Millennium Development Goals and now the Sustainable Developmental Goals (MDGs/SDGs) era, major challenges remain in reducing maternal and infant mortality worldwide. Though globally, maternal mortality rates have declined, the rate in Sub Saharan Africa with Ghana not being an exception is unacceptably high. Annual report from Komfo Anokye and Korle Bu Teaching Hospitals show that eclampsia results in the death of about eleven people every month at the Komfo Anokye Teaching Hospital and is the leading cause of hypertensive related deaths in the Korle Bu Teaching Hospital.[12]

The exact cause of pre-eclampsia is unknown. Angiogenic factors are known to aid in angiogenesis and are of extreme importance in normal pregnancy. A low PlGF is known to play a role in the pathophysiology of pre-eclampsia and not only related to the diagnosis of pre-eclampsia but may also predicts pre-eclampsia related adverse outcomes.[13,14] Management of the patient with pre-eclampsia depends on the time of presentation (early or late) and on the severity of the disease (severe or mild) and neonatal outcome when inadequate has many socio cultural effects on the family and society as a whole. Thus, this discovery of the role of PlGF in the underlying pathophysiology of placenta dysfunction resulting in pre-eclampsia has marked an important step for improving its early diagnostic and prognostic assessment.[15] There is the need therefore, to investigate if these markers can be used to enable clinicians decide on when to objectively deliver women with pre-eclampsia. Such a test would be invaluable to clinicians who may offer close follow-up and therapeutic interventions early in poor resource limited facilities. In Ghana, though some work has been done on proangiogenic factors like VEGF, there is limited data on the contribution of PlGF in the pathophysiology of pre-eclampsia.[9] In addition, there is paucity of data on its association with fetal outcomes. Determining the levels of PlGF in women at risk for pre-eclampsia can help decide whom to hospitalize and whom to send home especially in our setting where we have limited bed space available at our tertiary facilities. It also helps to identify pregnancies at high risk for immediate delivery and fetal and maternal outcomes.[16]

Perhaps performing a simple ELISA test to determine the angiogenic factor levels may be superior to serum uric acid in determining the severity of pre-eclampsia. If PlGF levels can be used to predict fetal outcome, it may be more beneficial than uric acid as performance of this test requires minimal expertise and less blood sample.[17] Delivery of women who have pre-eclampsia is done at the discretion of the obstetrician. In so doing, the neonate may be delivered late when the disease is thought to be seemingly under control but suffer the many complications associated with pre-eclampsia. There should be a standard guideline in place to determine when to objectively deliver women with pre-eclampsia to prevent neonatal complications. Such a guideline is not in existence thus this study

sought to bridge this gap in knowledge. This study examined the association between serum PlGF and PE and their effect on pregnancy and fetal outcomes.

Methods

Study design and site

This was a case-control study done from September, 2018 to February, 2019 (5 months' duration) at the Obstetrics and Gynecology department of the Korle-Bu Teaching Hospital in Accra, Ghana. Patients diagnosed with pre-eclampsia (cases) were recruited and followed up to 24 hours after delivery to see the trend of the PlGF levels and their relationship to fetal complications in comparison with normotensive pregnant women (control).

Study participants

The study included pregnant women between the ages of 18 to 45 years who gave informed consent to participate. Normal pregnant women with no complication were gestationally matched to women with pre-eclampsia and recruited from the Obstetrics and Gynecology department of the Korle Bu Teaching Hospital. Patients who had a history of chronic hypertension and/or other diseases were excluded.

Sample size determination

Sample size was calculated from SourceForge.net and the number of pairs of fifty was obtained giving a total sample of one hundred. For which we allowed 20% addition to count for incomplete data and lost to follow-up.

Data collection

The participants were interviewed using well-structured questionnaire to determine their demographics, knowledge of pre-eclampsia and complication of pre-eclampsia after a written informed consent. Maternal complications as diagnosed by the obstetrician and laboratory test results were obtained from the medical record book. Neonatal outcomes including APGAR scores-at 1 and 5 minutes after birth, birth weight and placenta weight measured using a digital scale were recorded. Length of neonate was measured using an infantometer with the baby flat on its back and legs extended and measured from top of the head to the heel of one foot. Chest circumference of neonate was measured to the nearest 1 mm at the level of the nipple line. Head circumference of neonate was measured to the nearest 1 mm using the occipital and frontal regions as marks. Gestational age was calculated using ultrasonography done during the first trimester. Stillbirth was recorded as an APGAR score of 0 at one and five minutes. Neonatal death was defined as any death within the first 28 days of life. The information on neonatal death was obtained by telephone calls to the mother on day 29 following birth. Participants had their blood pressure measured upon presentation to the clinic to and after resting for 10 minutes to determine if they had pre-eclampsia. The blood pressure was taken using the Omron digital sphygmomanometer. Once the diagnosis was established and patients consented, they were recruited into the study.

Laboratory measurement

Six milliliters (6mls) of blood was drawn from the antecubital fossa using a 10mls syringe for all patients and controls. This was then divided such that 3mls was poured into the ethylene diamine tetracetic acid (EDTA) vacutainer and the remaining 3mls poured into the serum for gel separator vacutainer and labelled with indelible ink. The blood was then stored for haematological and chemistry analysis. A second sample of blood was taken into the EDTA bottle during delivery and a third sample taken 24 hours after delivery. The blood was mixed gently to avoid hemolysis. Samples which were haemolyzed were rejected. After the blood was taken, it was allowed to clot by leaving it undisturbed at room temperature. The clot was removed by spinning the sample at a centrifugal speed of 1000xg for 10 minutes. The resulting supernatant (serum) obtained was carefully removed using a Pasteur pipette into clean Eppendorf tubes apportioned into 0.5ml aliquots and then packed in cryoboxes. Repeated freeze-thaw cycles was avoided and the serum obtained was stored in the freezer at the department of Physiology at -25 degrees and later transported to a laboratory at the Noguchi Memorial Institute for

Medical Research (NMIMR) on dry ice once all the samples were obtained.

The PlGF ELISA kits were used strictly following manufacturer's protocol. The sandwich ELISA technique was used and both case and control were run together to avoid bias. The samples were measured in duplicate and the mean values of individual samples were used for statistical analysis. Measurements included haemoglobin levels, total WBC counts and platelet count. Blood, urea, electrolyte, creatinine, uric acid, AST, ALT and albumin were measured using Hitachi 917 automated analyzer (Roche Diagnostics, Indianapolis, IN). The Sodium Meta Bisulphite method was used to detect sickle cell disease following strict quality assurance procedures.

Mid-stream urine was collected into clean dry plastic containers by the participants and the presence of urine protein was determined using the dip stick qualitative method according to manufacturer's instructions.

The colour of the dipstick changed from yellow to various shades of green depending on the amount of protein in the urine and was graded as follows: 1+, 0.3 gram per litre; 2+, 1.0 gram per liter; 3+, 3.0 gram per litre; 4+, greater than 10 grams per litre. Human placenta growth factor (PGF) was purchased from R&D Systems in Minneapolis, Minnesota, USA. The test principle applied in this method was Sandwich enzyme immunoassay. The concentration of PlGF in the sample was then determined by comparing the optimal density of the samples to the standard curve according to manufacturer's instruction. The EDTA anticoagulant whole blood was mixed very well and the manufacturer's instruction for operating the haematology analyzer was strictly adhered to.

Data analysis

Data was entered into an excel spreadsheet and analyzed using Statistical Package for Social Sciences version 20 (SPSS). Continuous variables were tested for homogeneity of variances using the Levene's test and for normality using the Shapiro Wilk test before analysis. The results were presented as mean ± SD. All of the variables were normal except the PlGF levels and placenta weight, thus these were reported using the median value. The median serum levels of PlGF between the normotensive pregnant and pre-eclamptic women were also compared using non-parametric Mann-Whitney U test and results presented using box plot and line graph. The pre-eclamptic group was, further, sub- classified into early- and late-onset pre-eclampsia. The Wilcoxon test was used to compare significance between paired data such as the levels of PlGF before and after delivery. Categorical data such as marital status, highest level of formal education, mode of delivery, cigarette use and knowledge of pre-eclampsia was expressed as proportions. Any probability ($p<0.05$) was regarded as statistically significant. The study was approved by the Ethical and Protocol review committee of the College of Health Sciences, University of Ghana (protocol identification number: CHS-Et/M. B-P2.14/2017-2018). Informed consent was obtained for all enrolled women. Participants got all their laboratory tests done at no cost to them and this was kept in their folders for future reference.

Results

The mean age of the case and control were (30.0 ± 6.1) years and (31.3 ± 4.4) years respectively, mean number of pregnancies including index pregnancy were 3 ± 2 same in both groups and the mean weight and height for the cases were (74.8 ± 18.5) kg and (1.6 ± 0.1) m while that for the controls were (77.1 ± 1.8) kg and (1.6 ± 0.1) m respectively. The demographic characteristics were similar between the two groups indicating that participants in the two groups were comparably homogeneous. Of the cases, 53 (88.3%) were either cohabiting or formally married while 7 (11.7%) were single. A similar trend was noticed in the controls where majority of them were either married or cohabiting (98.3%). Four women had a previous history of pre-eclampsia (2 in each group). The mean number of antenatal visits for the case was 6 ± 2 while that for the control was 5 ± 2. Prior knowledge on pre-eclampsia was generally low in both groups; 8/120 (6.7%), 6 of which were from the control group. All participants denied smoking.

Maternal systolic and diastolic blood pressures, mean arterial blood pressures and gestational age at delivery were all significantly different between cases and controls (Table1).

Table 1 Clinical characteristics between pre-eclampsia and control

Variable	Pre-eclampsia	Control	p-value
	Mean ± SD (N=60)	Mean ± SD (N=60)	
sBP diagnosis (mmHg)	171.3 ± 17.4	109.0 ± 11.3	<0.001*
dBP diagnosis (mmHg)	109.2 ± 12.4	67.3 ± 9.6	<0.001*
GA diagnosis (weeks)	34.7 ± 3.9	33.4 ± 3.7	0.062
MAP diagnosis	129.9 ± 12.9	81.2 ± 9.3	<0.001*
GA delivery (weeks)	35.0 ± 3.8	38.7 ± 1.4	<0.001*

Abbreviation: SD, standard deviation; sBP, systolic blood pressure; dBP, diastolic blood pressure; MAP, mean arterial pressure; GA, gestational age; *, Significant at p<0.05

Fifteen of the cases had maternal complications such as HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, placenta abruption, intrauterine fetal death and post-partum hemorrhage (PPH). Two women in the control group had Postpartum Haemorrhage (PPH), 45/60 (75%) of cases had caesarean section delivery as compared with 23/60(38.3%) of controls. All mothers were discharge home satisfactorily.

All neonatal parameters including weight and APGAR scores were significantly lower in cases than in controls (p<0.001) (Table 2).

Table 2 Comparing neonatal outcomes between pre-eclampsia and control

Variable	Cases	Control	p value
	Mean ± SD (N=60)	Mean ± SD (N=60)	
Birth weight (kg)	2.3 ± 0.9	3.3 ± 0.5	<0.001*
APGAR at 1 minute	6.1 ± 2.2	7.3 ± 1.2	<0.001*
APGAR at 5 minutes	7.2 ± 2.4	8.6 ± 0.8	<0.001*
Placenta weight (g)	315.5 ± 55.6	455.9 ± 72.1	<0.001*
Head circumference (cm)	31.9 ± 3.4	35.0 ± 1.9	<0.001*
Chest circumference (cm)	29.3 ± 4.0	34.0 ± 2.0	<0.001*
Total length (cm)	46.2 ± 6.1	51.0 ± 3.0	<0.001*

Abbreviations: SD, standard deviation; APGAR, appearance pulse grimace activity respiratory score; *, Significant at p<0.05

The median serum level of PlGF at diagnosis and during delivery in the case group was [78.7pg/ml, interquartile range (IQR: 46.9-188.7)] and [65.1pg/ml, IQR: 41.7-105.3)] respectively, while the level in the control group was [1,016.7pg/ml, IQR: 2, 80.7-2,177.2)] and [202.6pg/ml, IQR: 84.8-418.6)], p<0.001 (Figure 1).

There was a significant fall in the PlGF levels before and after delivery in both case and control p<0.001 and p=0.041respectively.

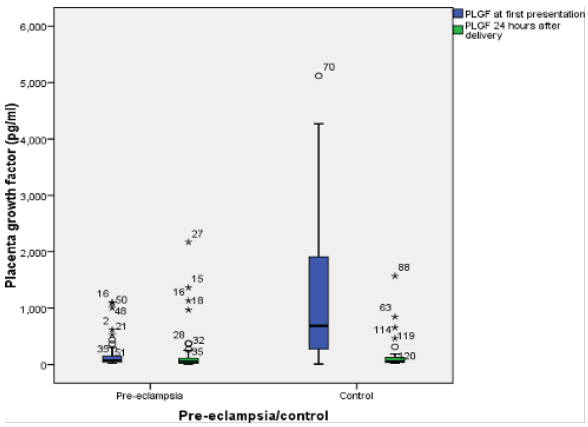

Figure 1 Maternal serum levels of PlGF at diagnosis and 24 hours postpartum

The median PlGF level in the cases group before delivery [68.7pg/ml; IQR: 44.5-161.0)] was significantly lower than in the control group [715.7pg/ml (IQR: 280.7 - 1,933.3)], p<0.001. However, the PlGF levels in both groups 24 hours' postpartum was not significantly different [52.9pg/ml (IQR: 20.0- 114.3)] and [59.7pg/ml (IQR: 30.8- 127.1) respectively, p=0.386 (Figure 1).

PlGF levels correlated with birth weight and NICU admission but not with stillbirth and early neonatal death. The mean PlGF level was significantly lower in neonates with low birth weight and those sent to NICU (Table 3). Twenty-seven (45%) of cases had neonates with low birth weight and 21 (35%) had their neonates admitted to the NICU. However, none of the babies of controls had low birth weight or admitted to NICU.

Table 3 Relationship of angiogenic markers with birth outcomes

Variables	Median PlGF	P-value
Low birth weight		0.032*
Yes	55.2 (IQR:41.0-89.4)	
No	79.1(IQR:57.6-79.1)	
NICU admission		0.004*
Yes	51.7 (IQR: 34.5-79.3)	
No	79.1(IQR:57.0-298.1)	
Stillbirth		0.736
Yes	54.4(IQR: 30.3-366.0)	
No	68.9 (IQR: 45.3-148.7)	
Early neonatal death		0.061
Yes	45.3(IQR:27.9-58.2)	
No	75.7 (IQR:50.9-166.8)	

Abbreviations: SD, standard deviation; *, Significant at p<0.05; NICU, Neonatal intensive care unit.

There were 7 (11.7%) early neonatal deaths in the case group but none in the control group and a positive correlation (R=0.50) was noticed between PlGF and placenta weight at a significance of 0.023 (Figure 2).

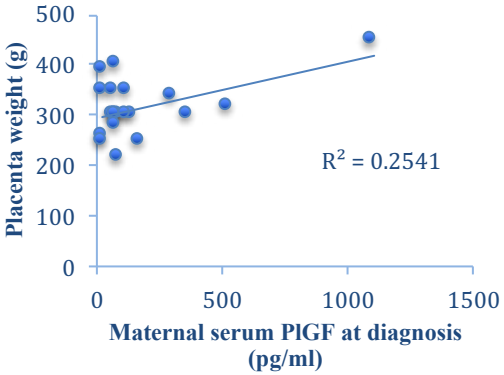

Figure 2 Correlation between placenta weight and maternal serum PlGF levels in women diagnosed with pre-eclampsia.

A positive correlation (R=0.50) was noticeable. The variation in the placenta weight that was explained by the PLGF level was 25.4%.

Discussion

The focus of this research was to determine the association between the angiogenic factor PlGF and neonatal outcomes in Ghanaian pregnant women. Even though both cases and controls were overweight, the BMI of controls was higher than that of the cases. This is in disagreement with a study which found the BMI of women diagnosed with pre-eclampsia to be higher than that of obese non pre-eclamptic pregnant women.[18] However, on further assessment of the study, it was realized that the authors compared the BMI of these women before they were diagnosed with pre-eclampsia. This study assessed and compared the BMI between cases and controls when they were diagnosed with pre-eclampsia. The cases may have had conditions such as hyperemesis gravidarum or anorexia earlier in their pregnancy which could have resulted in a smaller weight and corresponding smaller BMI.[19]

The mean arterial pressure was not surprisingly higher in the women diagnosed with pre-eclampsia as the definition of pre-eclampsia requires elevated blood pressures.[2] None of the women admitted to smoking. This was expected because generally in Ghana, the prevalence of smoking is low.[20]

In this study, it was observed that the PlGF level was generally lower in cases compared to controls. This is consistent with studies which showed that women with pre-eclampsia have lower circulating maternal serum levels of PlGF.[21,22] This reveals the importance of PlGF in the maintenance of endothelial cell health during pregnancy.[23] Findings from this study showed that PlGF was positively correlated with placenta weight. This supports the hypothesis that low PlGF is an indicator of abnormal placentation. This is shown by the significantly reduced placenta weights in cases than controls. Research has showed that women with pre-eclampsia tend to have infants with low APGAR scores.[24] There was a significant difference in birth weight of neonates between cases and controls with a p-value less than 0.001. This confirms that low birth weight is common in women with pre-eclampsia. PlGF levels had positive correlation with birth weight which confirms what is found in literature and shows how PlGF can serve as a guide to the birth weight.[25] If we can find a way to increase the PlGF levels, we could potentially increase the birth weight of babies with pre-eclampsia. In this study, neonates were delivered at an average gestational age of 35 weeks in cases and 39 weeks in the controls. The mothers whose neonates survived and were admitted to NICU had the PlGF level about one and a half times lower as compared to neonates who were not admitted to NICU. A similar trend was seen in those who had early neonatal deaths and those who had low birth weight. The PlGF level in women who had stillbirth was lower than those who did not have stillbirth in concordance with a study done in Canada, New Zealand and the United Kingdom which associated stillbirth to low PlGF levels.[11]

A strong positive relationship was seen between PlGF and placenta weight in controls. Clinicians can use this to their advantage with the view that if PlGF is not rising as steadily as it should or worse, if it is falling, then there is placental insufficiency. The obstetricians should therefore, aim at early delivery. Considering that the placenta is the main source of nutrients for the fetus, it may mean that the fetus might not be getting enough nutrients resulting in intrauterine fetal growth restriction. PlGF was seen to be about ten times lower in those who had pre-eclampsia. Similarly, its level was significantly low in those who had neonatal death and in those mothers whose neonates were admitted to the intensive care unit. This is possibly because the placenta which is the main source of nutrients to the fetus might be malfunctioning thus the fetus is already in danger in utero resulting in neonatal death. The low levels of PlGF also reflected the frequent admission to NICU.

A study done by Habli et al about a decade ago showed that patients with pre-eclampsia who were delivered between 35 and 37 weeks' gestation still had a higher rate of NICU admissions[21]. This probably means that as the disease advances, the neonatal outcome worsens hence the need for early delivery with possible guidance of PlGF levels and NICU admissions. Thus if Ghana, or for that matter developing countries can increase their NICU facilities and provide necessary medications like steroids which can be given before delivery or surfactant which can be given after delivery in anticipation of respiratory complications that may occur in the neonate, neonatal mortality can be reduced.

Conclusion

The study showed that women with pre-eclampsia are more likely to have neonates with low birth weights, early neonatal deaths and frequent admission into NICU. Women with pre-eclampsia have low PlGF level than those who had uncomplicated pregnancies. The findings of this study indicate that pre-eclampsia could influence neonatal outcome significantly. Constant monitoring of maternal PlGF levels, together with clinical signs and symptoms, may improve neonatal outcome.

Competing interest

The authors declare they have no competing interest.

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