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ORIGINAL ARTICLE

Placental Growth Factor in First Trimester of Pregnancy for Prediction of Maternal and Perinatal Adverse Outcomes

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Abstract

Purpose of the study Placental growth factor (PLGF) is an angiogenic factor in pregnancy. To find out correlation of plasma levels of placental growth factor in first trimester of pregnancy in Indian women who develop maternal and perinatal adverse outcomes was the aim of the study.

Methods A prospective longitudinal noninterventional study was done in the department of Obstetrics and Gynecology after obtaining ethics approval. After enrolling patients in the first trimester (11 weeks to 13 weeks 6 days), a questionnaire was filled for demographic characteristics. Uterine artery doppler was done for every patient and blood sample (5 ml) was taken by venu puncture of median cubital vein. Serum levels of PLGF were measured by enzyme linked immunosorbent assay using Thermo Scientific Pierce Human PLGF kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Patients were followed for their whole antenatal period and delivery outcomes.

Results Incidence of preeclampsia in our study was 9.3% (15/161) and fetal growth restriction (FGR) was 19.8% (32/161). Neither BMI nor nulliparity was found to have statistically significant correlation with development of preeclampsia. However, history of preeclampsia was found to be significant risk factor for prediction of preeclampsia (p value < 0.04). Plasma levels of PLGF were significantly lower in preeclampsia and FGR group and this difference was statistically significant (p value < 0.04). 7.5% still born occurred in complicated group and 10% needed NNU/NICU admission in this group.

Conclusion Measuring PLGF levels in first trimester of pregnancy can help in prediction of preeclampsia and FGR.

Keywords Placental growth factor · Preeclampsia · Fetal growth restriction

Introduction

Development of placenta is a complex mechanism which includes rapid development of angiogenesis, trophoblast invasion and adaptation of maternal and placental vascular dynamics. These all changes are prevalent between 8 and 12 weeks. So, it is logical to find out serum markers which can indicate about placental pathology in first trimester of pregnancy to predict adverse maternal and fetal outcomes. Strategy to develop effective first trimester screening will help to allow resources to be wisely used for prediction of several diseases and their management. If only maternal risk factors are taken into account, their sensitivity for preeclampsia prediction is only 45% [1]. If maternal mean arterial pressure is also added, sensitivity increases to 62.5% with reduction of false positivity [2]. Using uterine artery doppler, has good negative predictive value (97.5%) for prediction of preeclampsia [3]. Placental growth factor is an angiogenic factor which belong to vascular endothelial growth factor (VEGF) family. In normal pregnancy, PLGF increases in first and second trimester of pregnancy and reaches to its peak at 28–32 weeks of pregnancy [4, 5]. In complicated pregnancies levels of PLGF are low and does not increase with increasing gestation. It is plausible that evaluation of

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placental growth factor (PLGF) in first trimester can be added in first trimester screening with increasing sensitivity and decreasing false positivity for prediction of diseases like preeclampsia and fetal growth restriction. There is lack of data from India for PLGF first trimester studies. The aim of this study was to find out correlation of plasma levels of placental growth factor in first trimester of pregnancy in Indian women who develop maternal and perinatal adverse outcomes.

Material and Methods

This was a prospective longitudinal noninterventional study which was done in department of Obstetrics and Gynecology**,** King George Medical University, Lucknow in duration of one year period. Ethical clearance for the study was taken from the research cell of university. (ECR/262/ Inst/ UP/2013, letter no 1656/ethics R cell -17). Informed written consent was taken from all the patients before recruitment in the study. All patients coming to the antenatal outdoor of our department in the first trimester were enrolled for the study with inclusion criteria of singleton pregnancies between 11 weeks and 13 weeks 6 days gestation, patients with high risk of developing preeclampsia and fetal growth restriction based on clinical history and willing to participate in the study. Patients not willing to give consent, anomalous foetus in first trimester, threatened abortion in first trimester, twin pregnancy, pregnancy with heart disease, pregnancy in HIV positive patient were excluded from the study. The eligible candidates were explained about the study. Total of 180 women were enrolled for the study. A questionnaire was filled for every patient for demographic characteristics which included maternal age, body mass index (BMI), socioeconomic status, parity (parous or nulliparous), method of conception (spontaneous, ovulation induction or In vitro fertilization), personal history of chronic medical illness in terms of chronic hypertension, diabetes, systemic lupus erythematosus, antiphospholipid antibody syndrome chronic kidney disease, hypothyroidism etc. past history in terms of development of preeclampsia or fetal growth restriction (FGR), family history of Preeclampsia or FGR etc. Mean arterial pressure was calculated and the first trimester ultrasound was done using 3–7 MHz transabdominal curvilinear probe of Toshiba Xario Prime ultrasound machine. Ultrasound included confirmation of dating, singleton pregnancy, nuchal translucency measurement and uterine artery doppler. For Uterine artery doppler, sagittal section of uterus was obtained. Cervical canal and internal cervical os was identified. The transducer was gently tilted from side to side. Color flow mapping was used to identify each uterine artery along the side of cervix and uterus at the level of internal os. Pulsed wave was used with sampling gate set at 2 mm to cover the whole vessel and angle of insonation was less than 30 degrees. After getting three similar waveforms, PI was measured and mean PI of the left and right arteries was calculated. Blood sample (5 ml) was taken from each patient at 11 weeks – 13 weeks 6 days of gestation by venu puncture of median cubital vein. It was allowed to clot and serum was separated by centrifugation at room temperature. Serum samples were stored at −20 degree. Serum levels of PLGF were measured by enzyme linked immunosorbent assay (ELISA) using Thermo Scientific Pierce Human PLGF kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The ELISA kit used was based on sandwich technique which is used to identify a specific sample antigen. After nonspecific binding sites were blocked using bovine serum albumin, the antigen containing sample was applied to the plate. A specific primary antibody was then added that "sandwiches" the antigen. Enzyme-linked secondary antibodies were applied that bind to the primary antibody. Unbound antibody–enzyme conjugates were washed off. A substrate was added which was enzymatically converted to a color that can be later quantified. Reagents and standards in the kit were prepared following the standard protocol provided with the kit. 100 µl (µL) of standards and samples were pippeted into micro-ELISA plate wells, covered, sealed and incubated for 2.5 h at room temperature (RT). Liquid from each well was removed and 350 µL wash buffer was added to each well and washed 4 times with wash buffer. 100 µL of biotinylated antibody was added to each well. The plate was covered, sealed and incubated for 1 h at RT. Liquid from each well was removed and the plate was washed for 4 times. 100 µL of streptavidin HRP reagent working solution was added into the wells, covered, sealed and incubated for 45 min at RT. Liquid from each well was removed and the plate was washed for 4 times. 100 µL of TMB Substrate was added to each well, covered with plate sealer and incubated in dark for 30 min at RT. 50 µL of Stop Solution was then added to each well and immediately the absorbance of the colored product generated by the bound, enzyme-linked detection reagents was measured spectrophotometrically using an ELISA plate reader at an optical density of 450 nm. A standard curve was generated by plotting the average absorbance obtained for each standard concentration on Y axis and the corresponding PLGF concentration on X axis. The amount of PLGF in each sample was determined by interpolating from the PLGF concentration (X axis) to the absorbance value (Y axis). The values were expressed in pg /ml. After this, all women were followed in antenatal clinic throughout their pregnancy, and also in postpartum period. Women were monitored for maternal complications like development of preeclampsia, fetal growth restriction and perinatal outcomes. Outcome measures were defined as preeclampsia by the definition quoted by international society of study of hypertension as gestational hypertension,

Table 1 Demographic profile of two groups

	Group who developed preeclampsia or FGR (Group A) $n=40$	Group who did not develop preeclampsia or FGR (Group B) $n=121$	*P* value
Age (years) Mean ± SD	25.5 ± 3.3	26.2 ± 3.7	0.30
Body mass index (Kg/m^2)	23.8 ± 4.6	23.3 ± 4.4	0.12
Socioeconomic status			
Upper	3	13	0.52
Middle	22	70	0.70
Lower	15	38	0.47
Nulliparity	10	51	0.05

blood pressure more than 140/90 mm Hg accompanied by one or more of the following new-onset conditions at or after 20 weeks' gestation: Proteinuria, Another maternal organ dysfunction, including: Acute kidney injury (AKI) (creatinine ≥ 90 µmol/L; 1 mg/dL), liver involvement (elevated transaminases e.g., ALT or AST > 40 IU/L) with or without right upper quadrant or epigastric abdominal pain) neurological complications (examples include eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata), thrombocytopenia—platelet count below 150,000/µL, DIC, haemolysis), Uteroplacental dysfunction (such as fetal growth restriction, abnormal umbilical artery Doppler wave form analysis, or stillbirth). Fetal growth restriction (FGR) was defined as effective fetal weight or abdominal circumference less than tenth percentile for that gestational age and small for gestational age babies were defined as new-borns birthweight less than tenth percentile for gestational age. Results were analyzed by using SPSS version 21 for statistical analysis.

Table 2 Comparison of PLGF and uterine artery PI in both the groups

	Group A	Group B	*P* value
PLGF (pg/ml)	1.32 ± 0.57	7.39 ± 17.5	< 0.04
Uterine Artery PI	2.01 ± 0.13	1.43 ± 0.18	< 0.0001

Table 3 Comparison of pregnancy outcome in both the groups

Variable	Group A ($n=40$)	Group B ($n=121$)
Vaginal delivery	22	72
Caesarean section	18	49
Gestation for termination		
< 34 weeks	7	11
34–37 weeks	29	22
> 37 weeks	4	88
Fetal weight Mean (kg)	2.6 ± 0.69	2.8 ± 0.52
Intrauterine death	3 (7.5%)	0
NNU/NICU admission	4 (10%)	10 (8.2%)

Results

Out of 180 enrolled women, results could be analyzed for 161 women. 1 woman expired at 7 months gestation due to swine flu, 7 blood samples were hemolyzed and 11 women were lost to follow up. Out of 161, 15 women developed preeclampsia and 146 were normotensive throughout their pregnancy. So, incidence of preeclampsia in our study was 9.3%. Out of 15 women, 12 had non severe preeclampsia and 3 had severe preeclampsia. One patient developed severe preeclampsia at 32 weeks and two at 30 weeks of gestation. One patient became eclamptic and one had postpartum acute renal failure. Out of 161 women, 32 women had fetal growth restriction. So, incidence of FGR was 19.8%. 7 women were out of preeclampsia group who had associated FGR. So, out of 161 studied women, total 40 (24.8%) had poor maternofetal outcome in terms of preeclampsia and FGR (Group A). Group B had 121 (75%) women, who did not develop complications. Table 1 shows the demographic profile of all women. There was no difference in mean age of the patients who developed preeclampsia and FGR and who did not. Mean BMI was also found similar between the two groups. Maximum patients were from middle socioeconomic status from both the groups. Neither BMI nor nulliparity were found to have statistically significant correlation with development of preeclampsia. However, history of preeclampsia was found to be a significant risk factor for prediction of preeclampsia (p value < 0.04). Table 2 depicts PLGF values and explains that plasma levels of PLGF were significantly lower in preeclampsia and FGR group and this difference was statistically significant (p value < 0.04). Results of Uterine artery doppler PI done in first trimester of pregnancy, show that in preeclampsia patients uterine artery PI is significantly higher than normotensive group as shown in Table 2. (p value < 0.0001) Table 3 compares the delivery outcome between the two groups. 50% of the patients in the group

who developed preeclampsia or FGR needed termination between 34 and 37 weeks. There was no significant difference between the mode of delivery in the two groups. Mean birth weight was although more in uncomplicated group; however, it was not significantly different in between the two groups. 7.5% still born occurred in complicated group and 10% needed NNU/NICU admission in this group.

Discussion

In our study incidence of preeclampsia was 9.3%. The incidence of preeclampsia in India is found to be 4.6% by WHO secondary analysis in low- and middle-income countries [6]. India has an average of 4.5% reported preeclampsia cases as per data collected from individual institutions in Malik et al. study [7]. The reason for high incidence of preeclampsia in present study is that our institute is referral center and most pregnancies which are booked at our center are complicated and referred ones. The incidence of FGR in present study was 19.8% which is really high against available literature (5–10%) [8]. However a study from China showed incidence of FGR in Chinese population in tune of 15%. On analysis of FGR pregnancies in present study, it was found that maximum of these women was from medium to lower socioeconomic group, multiparous and anemic women. Association of FGR and preeclampsia in present study was 46.6% (7/15). In a study by Shen et al. 59.1% of severe preeclampsia had associated FGR which was comparable to present study [9]. However, a study from China showed association of FGR and preeclampsia only in 18.9%. This study has shown that with increasing severity of preeclampsia chances of FGR are increased and 22.4% of patients of severe preeclampsia had associated FGR [10].

Association of FGR with preeclampsia is explained by the fact that FGR is basically caused by endothelial damage resulting into hypoxia and acidosis and preeclampsia is associated with placental lesions. The more severe was preeclampsia, the earlier delivery was required and more was the chances of having small for gestational age as shown in study by Yu CK et al. [11] In their study the prevalence of SGA with PE was 82%, 47%, and 30% in those delivered at less than 34 weeks, between 34 and 37 weeks, and greater or equal to 37 weeks, respectively. The frequency of SGA in pregnancies without PE was 44%, 21%, and 8%, respectively. Body mass index and FGR are independent risk factors for preeclampsia recurrence. Even history of FGR may be a high- risk factor development of recurrent precalmpsia [12]. In present study BMI was not found a statistically significant risk factor for development of preeclampsia. However, Andrade et al. [13] found positive correlation between BMI and preeclampsia but this was not statistically significant, normotensive pregnancy (22.76 ± 4.39) versus PE (mild PE 26.14 ± 4.95 and severe PE 24.35 ± 3.08) ($p = 0.090$). In concordance to our study Calxito A.C. et al. [14] also did not find relationship between BMI and preeclampsia. However, Negin Rezavand et al. [15], in his regression analysis found, BMI to be a significant risk factor for development of preeclampsia but maternal age, parity and gestational age were not related with development of preeclampsia in their study also. Obesity (BMI > 30 kg/m^2) is found to be related to increase the risk of preeclampsia two to four times as shown in some studies [16–21]. Explanation about why obesity causes increased risk for preeclampsia is that low grade inflammation associated with obesity leads to endothelial dysfunction which in turn cause to develop preeclampsia.

In the present study, the patients who developed preeclampsia had lower PLGF than patients who had normal outcome and difference was statistically significant. This study supports that lowering of serum levels of PLGF in first trimester in complicated pregnancies can be used as a screening test for preeclampsia. This fact is supported in some studies [22–24] which also quotes that serum PLGF helps in prediction of preeclampsia in first and second trimester and helps in identification of candidates who will get benefit from aspirin. PLGF also predicts FGR because its levels were found low in first trimester in pregnancies which had FGR in present study. Benton SJ et al. [24] also found sensitivity of 93% and negative predictive value of 50% of PLGF as a marker for FGR. In present study, history of preeclampsia was found to be high-risk factor for development of preeclampsia and this was statistically significant. Boyd et al. [25] had also shown similar results in their study that previous early-, intermediate-, or late-onset preeclampsia increased the risk of recurrent preeclampsia with the same timing of onset 25.2 times (95% confidence interval (CI): 21.8, 29.1), 19.7 times (95% CI: 17.0, 22.8), and 10.3 times (95% CI: 9.85, 10.9), respectively, compared to having no such history. In present study fetal weight between two groups were different but it was not statistically significantly different. Takase et al. [26] in their study found no difference between the two groups normotensive and preeclampsia (3281 ± 229 and 3290 ± 100 gms, respectively); however, Calxito A. C. et al. [14] had found significant difference in fetal weight in preeclamptic (2602 ± 623 g) and normotensive patient (3100 ± 288 g) ($p < 0.001$). The strength of the study is that it has involved maternal history, uterine artery doppler and PLGF values for prediction of preeclampsia. Limitation is small sample size of the study. However, the present study concludes that among various high- risk factors, history of preeclampsia has strongest correlation for the occurrence of preeclampsia. Low values of serum PLGF in first

trimester may help in guiding the high-risk pregnancies for strict vigilance for development of preeclampsia and FGR complications.

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Declarations

Conflict of interest There is no conflict of interest.

Ethical Approval The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

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