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Low Serum Placental Growth Factor Levels in late second trimester can act as biomarker for predicting Intra Uterine Growth Retardation in pregnancies complicated by preeclampsia

Baixos niveis de PlGF no segundo trimestre da gravidez podem ser factores de predição de restrição de crescimento intra-uterino em gestações complicadas por pré-eclâmpsia.

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ABSTRACT

Background & Objectives: Preeclampsia is one of the commonest complications of pregnancy leading to IUGR. Placental Growth Factor is one of the many angiogenic factors which shows significant altered levels in preeclampsia compared to normal pregnancy. The present study aims to analyze whether estimation of serum PlGF levels in late second trimester can predict IUGR in pregnancies complicated by preeclampsia.
Materials and methods: A total of 150 nulliparous pregnant women admitted in antenatal wards or attending antenatal clinic were included in the study. They were divided into 3 groups; 30 women being normotensive and 60 each with diagnosed mild and severe preeclampsia respectively. Serum samples collected from the study groups were subjected to ELISA and PlGF level was calculated in all the samples. Birth weight of all the newborns were recorded.
Results: Mean serum PlGF Levels were found to be significantly low in mild and severe preeclampsia as compared to normal pregnancy. Total of 87 incidences of IUGR was recorded among 120 pregnant women with mild or severe preeclampsia in whom serum PlGF levels recorded at 26-32 weeks of gestation showed a sharp decline as compared to normal pregnant women. Cut-off value of serum PlGF levels for predicting incidence of IUGR in pregnancies complicated with preeclampsia were calculated statistically from analyzed data.
Conclusion: Estimation of serum PlGF levels at 26 weeks of gestation can be used as a screening test to identify women at risk for delivering IUGR babies with very high sensitivity, in nulliparous pregnant women with preeclampsia.

Key Words: Nulliparous, Preeclampsia, Serum PlGF, Angiogenic factors, ELISA, Screening test, IUGR.

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INTRODUCTION

IUGR (intrauterine growth restriction) is considered a severe complication in pregnancy leading to increased perinatal mortality as well as morbidity[1]. Among the various factors contributing to the development of IUGR, inappropriate placental development is the primary one. IUGR is thought to result from impaired trophoblast invasion of the maternal spiral arteries in early pregnancy, leading to reduced uteroplacental perfusion and placental hypoxia.[2] What causes the deficient trophoblast invasion remains unknown. There are however strong indications that low levels of angiogenic growth factors like PlGF (Placental growth factor) may be implicated.[2,3]

Placental growth factor (PLGF) is a polypeptide growth factor and is a member of the VEGF family, having 2 isoforms PlGF-1(smaller) and PlGF-2 (larger).[4,5] Expression of PLGF is limited to the placental tissue. PLGF binds with a high affinity to receptor tyrosine kinase Flt-1, which belongs to the VEGF family. Flt-1 is exclusively expressed on endothelial cells.[6,7]

Locally acting angiogenic factors like PlGF regulate angiogenesis and vascular transformation, the two most essential factors towards establishing placental blood circulation in a physiological environment of low oxygen tension so as to ensure proper placental development and a normal pregnancy outcome.[8] Plasma PLGF levels in pregnant women have been found to rise steadily throughout pregnancy from the level in non pregnant women(<50pg/ml) to levels exceeding 500pg/ ml after 30 weeks of gestation.[9] Serum PlGF level peaks at approx 26-30 weeks of gestation and then decreases as term approaches.[10]

Low PlGF levels in pregnant women is very commonly associated with preeclampsia and this has been documented in a number of studies.[11-17] Pregnancy induced hypertension (PIH), which includes preeclampsia, is one of the most common complications of pregnancy that can result in IUGR.[18]

The decrease in serum PLGF levels in preeclampsia with subsequent IUGR could be explained on the basis of the following hypothesis:

Preeclampsia is characterized by placental bed hypoxia, releasing sFlt-1 a receptor specific for angiogenic factors like PlGF. This results in functional deficiency of PLGF in circulation leading to inadequate development of the placenta and improper maturation of placental villi, which further leads to placental hypoxia. This **vicious cycle** goes on and on leading to placental damage and ultimately resulting in IUGR. (Please refer Fig.1)

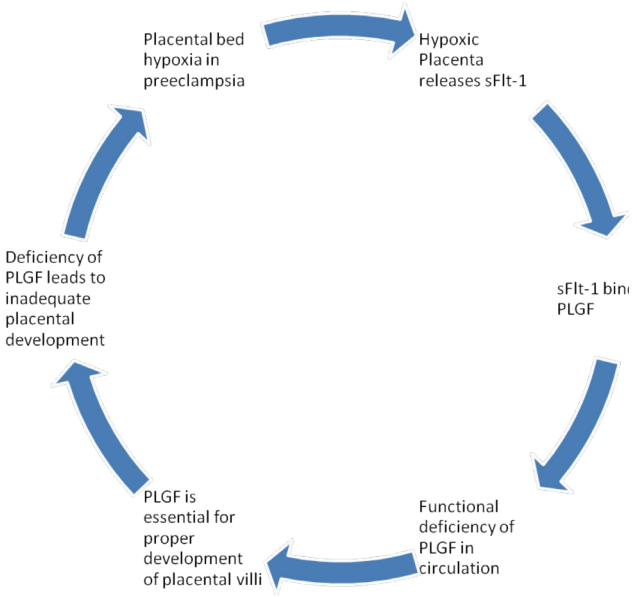

Figure 1: Low PlGF levels and Placental hypoxia occurring in a vicious cycle
1) sFlt-1: Serum Fms like Tyrosine Kinase
2) S.PlGF: Serum Placental Growth Factor

Most of the studies conducted so far regarding low PlGF levels and incidence of IUGR have taken the serum samples during the first trimester of pregnancy[19,20] or at delivery[21,22]. In one study sample collection was done during early second trimester.[23] Since serum PlGF levels in pregnant women reaches its peak during late second trimester (26-30 weeks of gestation)[10] hence it is suggested that low PlGF levels can be detected with higher sensitivity if sample collection is done during this period such that we can design a screening test to identify those women who are at risk to deliver babies with IUGR. Till date there has been only one study in which serum PlGF levels were estimated in late second trimester.[24] The aim of the present study was to compare serum PlGF levels in between normal pregnancies and those complicated by preeclampsia and also to investigate whether low serum PlGF levels in late second trimester can act as a predictor for the incidence of IUGR.

MATERIALS AND METHODS

The study was conducted in the Department of Anatomy in collaboration with the Department of Obstetrics and Gynecology, Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi. A total of 150 women were included in the study after taking a written consent. These were selected from pregnant women attending the antenatal clinic or admitted in the antenatal wards of the Department of Obstetrics and Gynecology.

Only nulliparous pregnant women with singleton pregnancies were included in the study. The study comprised of 30 normotensive, healthy pregnant women between 26-32 weeks of gestation who served as controls (group-1). 60 pregnant women with diagnosed early onset preeclampsia and 60 pregnant women with early onset severe preeclampsia (both between 26-32 weeks of gestation) were included in the study as group-2 & group-3 respectively.

Maternal age was recorded at the beginning of the study and pregnant women aged between 18-21 years of age were included in the study. Maternal height and weight were also recorded and those having a Body Mass Index in between 19-25 were included in the study(this particular range was chosen as normal BMI in Women is 18.5- 24.9 as per the latest data) . This was done to exclude maternal age and maternal weight, the two important confounding factors in the present study.(*This paragraph has been added as a part of revision*)

Inclusion Criteria

Mild preeclampsia was defined as blood pressure of at least 140/90 mm Hg or more (2 separate readings to be taken at least 6 hours apart) and proteinuria greater than 300 mg/24h or persistant 1+ dipstick proteinuria in random urine samples after 20 weeks of gestation.[25] Severe preeclampsia was defined as blood pressure of at least 160/ 110mm Hg and proteinuria of greater than 5gm/ 24h or persistant 2+or more dipstick proteinuria in random urine samples or the presence of persistant headache or blurring of vision or epigastric pain or reduced urine output(less than 400ml/ 24h) or platelet count less than 100,000/mm^3 or elevated ALT or AST or increased LDH after 20 weeks of gestation.[25]

Exclusion Criteria

Patients with history of chronic hypertension, diabetes mellitus, renal or cardiovascular disease, autoimmune disease, epilepsy, tuberculosis, bleeding disorder, congenital defects and drug intake were excluded from the study.

Detailed general physical examination and obstetric examination was done. Gestational age was confirmed with complete real time ultrasonographic examination. Venepuncture was performed and blood from the patients was collected to assess the levels of free PLGF by enzyme linked immunoassay (ELISA) technique. Serum samples were first collected at 26th gestational week and then followed up every week until the 32nd week of gestation. All the subjects were followed up in post partum period and birth weight of every newborn was recorded. Postnatal IUGR was defined as birth weight below the tenth centile corrected for gestational age.[26] No exclusion was made regarding the mode of delivery and women who delivered vaginally as well as those with a caesarian section were both included in the study. Newborns with any chromosomal or structural anomaly were excluded from the final analysis.

PLGF level was assessed in cases of preeclampsia and was compared to PLGF levels in control group subjects. The assay was done using DRG PLGF Enzyme Immunoassay kit which provides materials for quantitative determination of both the isomers of human placental growth factor (PLGF-1 and PLGF-2) in serum. The DRG PLGF ELISA Kit is a solid phase enzyme – linked immunosorbent assay (ELISA) based on sandwich principle.

Calculation of Results

The average absorbance value (optical density) was calculated for each of the standards and samples with the help of the Elisa reader. A standard curve was created by plotting the optical density for each standard concentration on the ordinate against the PlGF concentration on the abscissa. A best fit curve was drawn through the points of the graph. To determine the concentration of circulating PlGF for each sample, first the mean absorbance value was found on the ordinate and then a horizontal line was extended to the standard curve. At the point of intersection, a vertical line was extended to the abscissa and the corresponding PlGF concentration was read. Since no dilution of the samples was done during the assay procedure hence the actual concentration of PlGF was the same as the concentration read from the graph.

The serum PlGF concentrations between control and study groups were compared and p value was calculated using the independent students t-test. Correlation between Mean fetal birth weight of IUGR babies and mean serum PlGF levels in preeeclamptic women giving birth to IUGR babies was calculated using Karl Pearson's Correlation Coefficient. All the statistical evaluations were performed using SPSS (statistical package for social science) 16.0 version. Standard unimodal distribution curve was used for determining the cut-off values of PlGF for screening patients at risk of delivering newborn babies with IUGR with a very high sensitivity.

RESULTS

The mean serum PLGF level of Group2 (52.08±24.67) and 3 (10.48±5.96)was lower than that of Group1(265±192.69). This difference in serum PLGF concentrations were very

Table 1: comparison of serum PLGF in normotensive and preeclamptic women according to different gestational ages at sampling

Gestational age At sampling	S.PLGF in group1 controls (mean ± 2sd)	S.PLGF in group2 Mild Preeclampsia (mean ± 2sd)	S.PLGF in group3 Severe preeclampsia (Mean ± 2SD)
26-28 weeks	315.71±318.65	52.5±53.66	9±10.14
28-30 weeks	241.61±256.48	49.52±46.17	10.63±12.58
30-32 weeks	280±170.91	53.09±51.82	11.79±11.84
P value	P_1=.519 P_2=.821	P_1=.136 **P_2=.019***	P_1=.838 P_2=.750
	P_3=.692	P_3=.457	P_3=.688

Table 1: P1 denotes the statistical significance of statistical correlation between mean Serum PlGF levels in 26-28 gestational weeks and at 28-30 weeks across the 3 groups. P2 denotes the same between 26-28 weeks and 30-32 weeks. P3 denotes the same between 28-30 weeks and 30-32 weeks. P >0.05 is statistically not significant, P <0.05 is statistically significant(*), P <0.001 is statistically highly significant (**), P< 0.0001 is statistically very highly significant(***). Mean Serum PlGF values in each of the groups is represented as Mean± 2SD in order to ensure a very high sensitivity. (Foot Note Added as revision)

Table 2: incidence of IUGR & other fetal complications across different study groups

Indicators of adverse fetal outcome	Group1 controls n=30	Group2 mild PE n=60	Group3 severe PE N=60	p value
IUGR	0	32	**55**	**P_1=- P_2=<.0001*** P_3=<.0001***
Intra uterine death (IUD)	0	3	**5**	**P_1=- P_2=<.0001*** P_3=<.0001***
Fetal Distress	0	36	**55**	**P_1=- P_2=<.0001*** P_3=<.0001***
Preterm/Premature Babies	0	42	**55**	**P_1=- P_2=<.0001*** P_3=<.0001***

Table 2 : P1 denotes the statistical significance between Group1 and Group 2, P2 denotes the same between Group1 and Group 3, P3 denotes the same between Group 2 and Group 3. P >0.05 is statistically not significant, P <0.05 is statistically significant(*), P <0.001 is statistically highly significant (**), P <0.0001 is statistically very highly significant(***).
(Foot note added as part of revision)

highly significant (p<.0001). Also there was a significant difference between the serum PLGF concentration of Group2 and Group3, the concentration of PLGF being lower in Group3 as compared to Group2. This difference was also highly significant (p<0.0001) (Please refer fig no.2).

It can be concluded from Table 1 that though the differences between Serum PlGF levels between different gestational age groups in both mild and severe preeclampsia were not found to be statistically significant but the mean serum PlGF concentrations showed a sharp decline when compared between normal pregnancy, mild preeclampsia and severe preeclampsia and this difference was found to be statistically highly significant.

32 pregnant women with mild preeclampsia and 55 pregnant women with severe preeclampsia gave birth to newborn with IUGR, whereas none of the babies born to women with uncomplicated pregnancy were found to be suffering from IUGR (Please refer Table no.2). Hence it may be said that a total of 87 incidences of IUGR was recorded among 120 pregnant women with mild or severe preeclampsia in whom

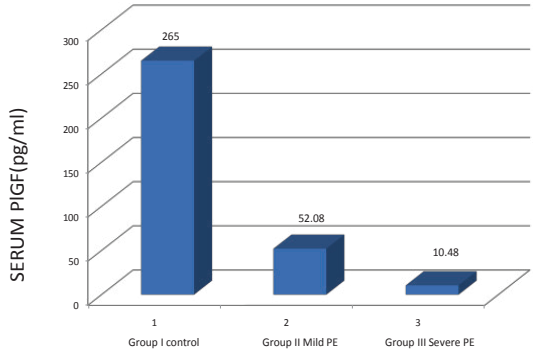

Figure 2: Comparison of mean serum PlGF levels in Normotensive and preeclamptic women
1) S.PlGF: Serum Placental Growth Factor
2) Pg/ml: Pico gram per milliliter
3) PE: Preeclampsia

serum PlGF levels recorded at 26-32 weeks of gestation showed a sharp decline as compared to normal pregnant women. Mean serum PlGF level in 87 recorded cases of IUGR was found to be 29.34±19.10 pg/ml (Mean ± SD), whereas the mean serum PlGF level in 30 recorded cases of normal uncomplicated pregnancy were 265±192.69 pg/ml (Mean ± SD)(Please refer fig no.2). Here serum PlGF values are mentioned as Mean ± SD, as we just want to show the mean value only within a range of single standard deviation.

Mean fetal birth weight of babies born with IUGR showed a negative correlation (r= -0.388) with Mean Serum PlGF level in preeclamptic women giving birth to IUGR babies and this correlation was found to be statistically highly significant (p< .0001).

Since almost 73% of pregnant women with low serum PlGF levels recorded in late second trimester delivered newborn babies with IUGR, hence it may be suggested that this particular biomarker has a high predictive value for the incidence of IUGR in pregnancies complicated by preeclampsia and can be used to design a screening test to identify at risk pregnant women. For this purpose the cut-off values of serum PLGF were calculated using the standard unimodal distribution curve. In order to ensure high sensitivity the cut-off point was selected in such a way so as to include 95% of serum PLGF level values.

Hence the range of serum PLGF values that would be able to predict the incidence of IUGR with high sensitivity during late second trimester(26-32 weeks of gestation) can be calculated as follows:

Mean serum PLGF levels in pregnant women who gave birth to newborn with IUGR ± 2 standard deviations (Mean ± 2SD):

29.43 ± 2 (19.10) = (- 8.77 - 67.63) pg/ml

Table 3: Correlation between Mean serum PlGF concentration in preeclamptic women giving birth to IUGR babies and Mean Fetal Birth Weight of IUGR babies.

Intrapartum Fetal complication	Correlation with serum PlGF levels in Preeclamptic women(r)	P-Value
IUGR	**-0.388**	**<.0001*** **

Table 3: The P- value denotes the statistical significance of the correlation obtained between the two factors. Correlation between the two factors was obtained using the Karl Pearson's Correlation Coefficient. P <0.05 is statistically significant(*), P <0.001 is statistically highly significant (**), P <0.0001 is statistically very highly significant(***). (Table 3 added as part of revision)

So it can be said that if the cut off value for serum PLGF in between 26 -32 weeks of gestation is set at <68 pg/ml then nulliparous pregnant women with preeclampsia who are at risk of delivering newborn with IUGR can be screened with maximum sensitivity.

In the present study:

a) None of the control women gave birth to IUGR babies but serum PlGF levels were found below cut off levels in 4 of them.
b) 32 women with mild preeclampsia gave birth to IUGR babies and 30 among these women had serum PlGF levels below cut off levels. 5 women in this group had serum PlGF levels below cut off but did not gave birth to IUGR babies.
c) 55 women with severe preeclampsia gave birth to IUGR babies however all the 60 women in this group had serum PlGF levels below cut off levels.

The sensitivity and the specificity of the screening test with serum PlGF levels using <68 pg/ml as cut off value was calculated as follows:

1) Total number of women in our study: 150
2) Total number of women who had serum PlGF level below cut off level and gave birth to IUGR babies : 85 (TRUE POSITIVE)
3) Total number of women who had serum PlGF level above cut off level and gave birth to IUGR babies: 02 (FALSE NEGATIVE)
4) Total number of women who had serum PlGF level below cut off level and did not gave birth to IUGR babies: 14 (FALSE POSITIVE)
5) Total number of women who had serum PlGF level above cut off level and did not gave birth to IUGR babies : 49 (TRUE NEGATIVE)

Sensitivity of the test: 85/ (85+2) x 100 = 97.7%
Specificity of the test: 49/ (49+14) x 100= 77.8%

DISCUSSION

In the present study we have tried to compare serum PlGF levels in between normal pregnancies and those complicated by preeclampsia and also to investigate whether low serum PlGF levels in late second trimester can act as a predictor for the incidence of IUGR. To the best of our knowledge this is the first case control study conducted on Indian population to investigate the role of low serum PlGF in the incidence of IUGR in pregnancy complicated with preeclampsia.

According to Torry et al (1998), serum PlGF level peaks at approx 26-30 weeks of gestation and then decrease as term approaches.[10] In our study serum PlGF levels follow an almost similar pattern, being highest at 26-28 weeks of gestation across all patient groups. There is a sharp decline in the levels of serum PlGF in patients with mild as well as severe preeclampsia compared to normotensive pregnant women from 26th week itself (refer table no.1). Thus fall in the serum PlGF levels in pregnancy complicated with preeclampsia is most evident during 26-30 weeks of pregnancy i.e. in the late second trimester. Utilising this data we have tried to design a screening test based on the estimation of serum PlGF to predict the occurance of IUGR among pregnant women with preeclampsia.

Lulla (2007) stated that in case of severe uteroplacental insufficiency no changes in the growth of the fetus or in the ultrasound takes place until 20-24 weeks of gestation and in case of mild uteroplacental insufficiency no changes takes place until 26-32 weeks of gestation.[27] Hence the authors may suggest that even if the screening is done on the 26th week of gestation that would allow for timely interventional measures to be taken in pregnant women at risk so as to ensure the birth a heafrlthy newborn.

Till date the most popular method for screening IUGR is serial ultrasound screening starting from 18 weeks of gestation.[27] But in developing countries like India where most of the population is rural this happens to be quite an expensive option. Hence we have taken up this project to design a screening test with a biomarker which would not only prove to be a cost effective option in developing countries but also convenient to patients because of just one time sample collection i.e. at 26 weeks of gestation.

When we recommend a screening test for a disease, 3 aspects are to be kept in mind:

- Prevalence of the disease in the population on whom the screening test is to be implied.
- Nature of the disease under study.
- The screening test should be cost – effective.

When the prevalence of the disease is high in a population group, the cut-off value is set at a lower level which will increase sensitivity. If the disease is very lethal and early detection can markedly improve prognosis, a greater degree of sensitivity, even at the expense of specificity is desired.[28]

Since the prevalence of preeclampsia is high and the disease is lethal by virtue of its complications if not diagnosed and treated early, so it is desirable that a screening test for preeclampsia should be highly sensitive. The present study has prescribed the cut-off values of serum PLGF for screening nulliparous pregnant women at risk of delivering newborns with IUGR(<68 pg/ml) such that high sensitivity is maintained. To the best of our knowledge this is the first study to have suggested a cut-off value for a serum biomarker (PlGF in this case) for predicting the incidence of IUGR in pregnancies complicated by preeclampsia.

The sensitivity of suggested screening test was calculated as 98%, which is very high. The specificity was calculated as 78%.

According to Table no.1 Mean serum PlGF level recorded in women with mild preeclampsia and severe preeclampsia were 52.5± 53.66 pg/ml (Mean ± 2SD) and 9.0 ± 10.14 pg/ml (Mean ± 2SD) respectively. Hence the maximum values in mild preeclamptic women were 106.16 pg/ml and 19.14 pg/ml respectively. The serum PlGF cut-off value that we have suggested at 26 weeks of gestation is <68pg/ml. Hence it can be concluded that going by the results of our study all pregnant women with severe preeclampsia will have the risk of giving birth to IUGR babies. But in case of women with mild preeclampsia, only those with serum PlGF levels <68 pg/ml will be screened to be at risk of giving birth to IUGR babies. In other words diagnosis of mild preeclampsia alone would not be sufficient to identify pregnant women at risk of giving birth to IUGR babies, it is only when their serum PlGF levels is below 68 pg/ml, that they would be at risk for delivering IUGR babies.

CONCLUSION

Preeclampsia being one of the most common complications in pregnancy that leads to IUGR , for quite some it has been a topic of research to identify pregnant women at risk of delivering IUGR babies. Till date there has been a traditional reliance on serial ultrasound, Doppler ultrasound etc. but the

problem remains as these procedures are costly and attrition rates are also low being a multiple step procedure.

Over the years many researchers have stressed the fact that there is a need to identify a potential biomarker for predicting the occurrence of IUGR in pregnant women such that timely intervention could be undertaken. The present study suggests the estimation of serum PlGF levels in nulliparous pregnant women at 26th week of gestation as a screening test with 98% sensitivity (very high) and 78% specificity to identify pregnant women at risk. Further studies on this biomarker could well prove to be the key to improving care for both the mother as well as the newborn in the future.

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