A Prospective Study of Placental Growth Factor Assay as a Novel Biomarker in Predicting Early-Onset Preeclampsia in High-Risk Patients

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Abstract

Introduction Preeclampsia is a leading cause of maternal and perinatal mortality and morbidity, but signs and symptoms are non-specific and may vary. The root cause is imbalance of circulating angiogenic factors of placental (syncytiotrophoblast) origin, with consequent low levels of

placental growth factor (PIGF) which may aid in diagnosis and prediction of disease.

Aims and Objectives To study the incidence of women at risk of developing early-onset preeclampsia by plasma placental growth factor biomarker assay in high-risk patients, to assess the maternal outcome in patients with PIGF values below cutoff for presenting gestational age, to calculate sensitivity, specificity, positive predictive value and negative predictive value of PIGF assay in predicting preeclampsia and to conclude whether PIGF biomarker assay can be an effective screening test in high-risk patients for prediction of early-onset preeclampsia.

Materials and Methods The study was carried out in the Department of Obstetrics and Gynecology at a tertiary care center. It is a prospective study, and study period extended

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from April 2012 to October 2013. One hundred pregnant women with 20- to 34-week gestational age with possible high risk of developing preeclampsia attending the antenatal clinics and high-risk OPDs were screened in the present study after explaining the nature of the study. PLGF concentration was quantitated using plasma and processed in Triage kit device (fluorescence immunoassay device). PLGF concentration was categorized against a specific range for specific gestation, and values below the range or <12 pg/ml were considered screened positives. The cases were followed up till delivery.

Results Twenty-two cases were screened positive, of which 20 developed preeclampsia with a strong positive prediction value of more than 90 %.

Conclusion PLGF is a strong predictor and a useful assay for early-onset preeclampsia.

Keywords Early onset preeclampsia · Placental growth factor · Angiogenic biomarkers · High risk pregnancy

Introduction

Preeclampsia and eclampsia are the most dreaded complications of pregnancy, and they affect both mother and fetus leading to high morbidity and mortality. Accurate prediction of adverse pregnancy outcomes has been desired for long in the field of obstetrics. Although they generally manifest later in pregnancy, their underlying pathophysiology is largely established early in pregnancy. This finding has sparked great interest in the search for tests to predict them early in pregnancy before these complications occur. However, diagnosis is based on traditional, unreliable and non-specific clinical markers, most commonly blood pressure and urinary protein excretion; both are subject to observer error and poor test accuracy for identifying women at risk of adverse outcome [1]. From the Preeclampsia Integrated Estimate of Risk (PIERS) study dataset [2], 254 of 2024 women with preeclampsia did not fulfill the research definition of preeclampsia on the day of their hospital admission. Women with non-proteinuria, preexisting or gestational hypertension can evolve into preeclampsia and have been found to have worse outcomes than their classically defined preeclampsia counterparts [3] and represent a clinically challenging subset of women to diagnose.

Preeclampsia can be considered a two-stage disease, of which poor placentation is the first stage. Current evidence suggests that the clinical (second) stage of preeclampsia is mediated, at least in part, by an imbalance of circulating angiogenic factors of placental (syncytiotrophoblast) origin. The proangiogenic marker, placental growth factor

(PIGF), circulates at a high concentration in normal pregnancy. In preeclampsia, there is increased release of its soluble receptor, soluble fms-like tyrosine kinase-1 (sFlt1), from the placenta in response to oxidative and inflammatory stress, which binds to circulating PIGF. As a consequence, circulating levels of unbound PIGF are decreased in women with preeclampsia. There is consistent evidence of a strong correlation between decreased levels of PIGF in pregnant women with preeclampsia [4]. PIGF and its antagonistic receptor, Fms-like tyrosine kinase-1 (Flt-1), have recently emerged as two potential angiogenic markers for preeclampsia [5–7]. Preeclampsia is associated with reduced maternal levels of PIGF and increased levels of soluble Flt-1 (sFlt-1) [8–10]. These markers appear to improve diagnostic accuracy because they are closer to the primary placental pathology. They have the potential to predict the onset of preeclampsia and increase the accuracy of a diagnosis when used in combination with traditional diagnostic markers. Early-onset preeclampsia is associated with greater changes in PIGF compared with late-onset preeclampsia and normal pregnancy [11–13].

While PIGF and sFlt-1 both change in preeclampsia (PE), reliance on both markers for a diagnosis may increase the probability for false-negative and false-positive results as there is inherent arithmetic instability in the ratio used; hence, sole PIGF level detection as a predictor is recommended and studied [14].

Plasma PIGF levels may seem preferable as biomarker, firstly because early fall in levels are seen in serum well before symptoms appear and secondly because a fair analysis of basic root defect can be done in high-risk cases. Lastly, atypical cases can also be predicted with same sensitivity.

Materials and Methods

The study was conducted as a prospective study including 100 high-risk pregnant women in a tertiary care hospital, and the study period extended from April 2012 to October 2013 after clearance from the ethical committee of the hospital.

Pregnant women attending the antenatal clinics and high-risk OPDs were screened for possible participation in the present study after explaining the nature of the study. A total of 100 women were included in the study.

Inclusion Criteria

Women between 20 and 34 weeks (wks) of gestation with one or more of the following risk factors and with willingness to share their contact number for further follow-up were enrolled.

High-Risk Factors

- Primigravidae,
- Primi/multigravidae with any of the following issues:
 - a. Medical disorders, for example essential hypertension, obesity, renal disorder, anemia,
 - b. History of PIH, preeclampsia, eclampsia in previous pregnancy,
 - c. Patients with associated obstetrics complications—twins, polyhydramnios.
- Autoimmune diseases, e.g., SLE, rheumatoid disease,
- history of documented PCOS,
- extremes of age
 - a. teenage pregnancy,
 - b. elderly gravida.

Exclusion Criteria

- Small for gestational age,
- IUGR cases,
- Any vaginal infection,
- Premature rupture of membranes.

Detailed menstrual, obstetric, past, family and personal history was taken. Thorough general, systemic and obstetric examination was done.

Gestational age (GA) at the time of study was recorded, and duration of pregnancy was confirmed by routine ultrasound and examination between 20 and 34 weeks. All women included in the study were investigated for blood grouping, Rh typing, blood examination (hemoglobin, total and differential count, platelet count, blood urea and random blood sugar) and urine routine and microscopic examination. Special investigations like liver and renal function test were carried out in hypertensive women. Venous plasma samples of women included in the study were collected in EDTA tubes. Samples were prepared by centrifugation and frozen at -80°C . Plasma was analyzed for PIGF using the Alere Triage PIGF assay according to the Manufacturer's instructions.

Reagents and Materials Provided

The test device contains all the reagents necessary for the quantification of PIGF in EDTA-anticoagulated plasma specimens [15].

The test device contains:

- Murine monoclonal antibodies against PIGF,
- Fluorescent dye,
- Stabilizers.

Procedure employs standard immunoassay techniques using recombinant murine antibodies to specifically and quantitatively detect the target protein and PIGF (placental growth factor) in EDTA-anticoagulated plasma specimens. The assay cartridge contains two different PIGF-specific monoclonal antibodies: One antibody is immobilized on a solid phase (capture zone), and a second is conjugated to fluorescent nanoparticles (detection).

The measurable range of the assay is 12–3000 pg/mL. Concentrations below 12 pg/mL are value assigned based on the calibration curve, but this value is displayed to the user as a qualitative result “<12 pg/mL.” A positive test was defined as a PIGF concentration <5th percentile for gestational age (GA) of normal controls. The distributions of PIGF concentrations within each GA period were characterized by percentiles as shown in the table below [16].

PIGF percentile (pg/mL)			
GA bin (in weeks)	5th %	50th %	95th
GA < 19	56.2	123	365
$19 \leq \text{GA} < 24$	62.9	154	452
$24 \leq \text{GA} < 29$	130	407	1296
$29 \leq \text{GA} < 32$	128	494	1460
$32 \leq \text{GA} < 35$	70.4	399	1406
$\text{GA} \geq 35$	14.6	53.9	327

Those cases with PIGF values below the cutoff value were taken as “screen-positive” cases.

A patient was diagnosed to have preeclampsia if there is a rise in systolic pressure of at least 30 mmHg or a diastolic of at least 15 mmHg over the previously known blood pressure or an absolute rise in the blood pressure of at least 140/90 mmHg with 24 h urine albumin >300 mgs or urine dipstick >+1.

Cases were followed in the antenatal clinic and screened routinely for the development of preeclampsia. The decisions regarding management were based solely on clinical symptomatology. Those with poor prognostic features of preeclampsia [17] were terminated at any time of gestational age in second or third trimester. Rest of the patients presented during labor only. Maternal outcome was assessed as clinical manifestations of preeclampsia and development of its various complications.

Results

The present study shows that 22 patients were “screened positive” with PIGF values below 5th percentile for respective gestational age, of which 20 patients (90.90 %)

developed PE, while 78 patients had PIGF values above 5th percentile, of which none developed PE later. Maximum number of screen-positive cases (45 %) belong to age group of 26–30 years, of which primigravidae was the most common risk factor (43 %). Other common known risk factors are shown in Table 1.

In present study, due to multiplicity of risk factors, assessment regarding number of associated risk factors was done. Thirty-one percentage of screen positives had a single high risk factor, 59.09 % had two high risk factors and 9.09 % had three or more associated high risk factors for PE.

In the current study as depicted in Table 2, maximum number of cases (44 %) were in the gestational age range of 32–34 weeks due to lack of antenatal visits and late reporting of patients. Maximum number of screen positives also belonged to the above mentioned group of cases (9 out of 22). All the screen positives progressed to develop preeclampsia in the age group of 19–23 and 32–35 weeks. An important observation was that 100 % of patients developing early-onset PE in the range of 19–29 weeks had additional comorbidities.

In the present study, 65 % high-risk cases delivered normally, 12 % cases delivered preterm, 8 % had preterm LSCS, 6 % had twin vaginal delivery, 1 % aborted, 1 % had hysterotomy and term LSCS was done in 7 % cases for obstetric indications.

Among screen-positive cases, as shown in Fig. 1, cases developing PE, 12 cases were delivered preterm, preterm LSCS (36 weeks) was done in 6 cases, one patient aborted and one had hysterotomy. Thus, maximum patients with PIGF values below cutoff levels delivered preterm or had LSCS.

Considering the perinatal outcome of the 26 fetuses in 22 screen-positive cases, 20 neonates were preterm live births, 2 fetuses (single case with twin pregnancy) aborted spontaneously, while 4 cases had intrauterine fetal demise.

Additional comorbidities were present in 15 out of 22 screen positives. Out of these 15 cases, 12 had a quantitative PIGF level of <12 pg/ml. This observation points to an inversely proportional relationship between quantitative values of the biomarker and poor maternal outcome.

Analysis

From Table 3, the present study shows that for prediction of PE, PIGF biomarker assay has the following indices:

Sensitivity	100 %
Specificity	97.5 %
Negative predictive value	100 %
Positive predictive value	90.90 %

Discussion

The current study is one of the pioneer studies to be carried out in the Indian subcontinent. The main focus has been to improve sensitivity of the predictive assay. The study analyses predict accuracy of serum placental growth factor assay in patients at risk of early-onset preeclampsia. The highest clinical sensitivity was obtained by using a variable cutoff, based on the 5th percentile of PIGF concentration in the reference pregnancies over a defined range of gestational ages. This addresses the normal changes in circulating PIGF level in pregnancy [18]. Cnossen et al. have highlighted the need for a test with high sensitivity in a setting with low resources and a high incidence. Thus, a greater preference has been given to minimize false negatives and to ensure appropriate resource use [19].

The PIGF concentrations were revealed to neither the obstetrician responsible for decision-making and

Table 1 Distribution according to risk factors

Risk factors	No. of patients	%	No. of screen positives	% of screen positives of risk factor
Primigravida	43	43	10	23.25
Anemia	38	38	6	15.7
Teenage pregnancy	11	11	3	27.27
Elderly gravida	3	3	1	33
Multiple gestations	10	10	4	40
H/O PIH in previous pregnancy	13	13	5	38.46
H/O HTN, DM, kidney disease	4	4	2	50
PCOS	2	2	2	100
Obesity	4	4	2	50
Primary infertility	2	2	1	50

Table 2 Distribution according to gestational age and screen positives

Gest. age	Cutoff (PIGF pg/ml)	No. of patients	No. of screen positives	%	No. of pts developing preeclampsia	No. of pts developing additional comorbidity
$19 \leq \text{GA} < 24$	56.2	11	2	9.09	2	2
$24 \leq \text{GA} < 29$	62.9	20	6	27.27	5	5
$29 \leq \text{GA} < 32$	130	25	5	22.72	4	2
$32 \leq \text{GA} < 35$	70.4	44	9	40.90	9	5

Fig. 1 Distribution of cases according to the mode of delivery in screen-positive cases who developed PE**Table 3** Statistical indices of the PIGF values in prediction of PE

	Cases who developed PE	Cases who did not develop PE	Total
PIGF screen positives	20	2	22
PIGF screen negatives	0	78	78
Total	20	80	100

management nor the patient until all diagnoses had been adjudicated, so that the test result could not influence decisions for delivery. The study has a few limitations. Firstly, it has a small sample size of 100 patients excluding those with loss to follow-up. More centers are currently being incorporated to perform this project at a larger scale and exclude any sample size related bias. Secondly, analysis is done by a single point of time assay of plasma PIGF which could lead to an increase in false negatives, although this phenomenon did not occur in the current study.

Test performance was evaluated as sensitivity, specificity, positive and negative predictive values using gestational age-dependent product insert cutoffs. Fixed cutoffs were not used, taking into consideration the variability of PIGF levels in pregnancy and hence to increase sensitivity of the assay [14].

Definitions and outcomes were prespecified in the study protocol. The primary analysis was of predictive accuracy of low plasma PIGF (<5th centile for gestational age) considering the need to deliver for preeclampsia within 30 days of testing in women at high risk of developing preeclampsia before 35-week gestation. The threshold of 35 weeks was chosen in view of normal fall in PIGF levels after about the middle of the third trimester which could increasingly diminish the differences between preeclampsia cases and normal ones and thus affect accuracy of Triage test. A further observation was noted that any case with PIGF levels <12 pg/ml had a grave prognosis and higher incidence of additional comorbidities.

Previous reports using angiogenic markers to diagnose and predict preeclampsia have demonstrated great potential. Using serum samples, Verloren et al. [20] found an AUC (area under curve) of 0.95 for all preeclampsia cases and 0.97 for early-onset preeclampsia. Knudsen et al. [21] found that clinical sensitivity of the Triage PIGF test for the pooled GA range of $21 < \text{GA} < 35$ using a gestational age-dependent variable cutoff is 1.0 with a corresponding clinical specificity of 0.94. Chappell et al. [4] conducted a large multicentric study and demonstrated that PIGF < 5th centile had high sensitivity (0.96; 95 % confidence interval, 0.89–0.99) and negative predictive value (0.98; 0.93–0.995) for preeclampsia within 14 days; but specificity was lower (0.55; 0.48–0.61). In addition, they did risk stratification and a time to delivery analysis as well. Benton et al. [14] did a comparative analysis of two commercially available biomarker assays and reported that Triage PIGF assay had a sensitivity 100 % [86–100] and specificity of 96 % [85–99] while Elecsys sFlt-1/PIGF had a sensitivity of 64 % [43–82] and specificity of 100 % [93–100]. They also inferred an AUC of 0.99 for gestational age at diagnosis <35 weeks for both the Alere Triage PIGF and Roche Elecsys sFlt-1/PIGF ratio, evidence that the single biomarker, PIGF, has the same diagnostic performance as the ratio of the two biomarkers. The measurement of a single biomarker simplifies interpretation of the test result and would reduce the cost of testing [21].

The parameters achieved by the current study have the highest sensitivity and specificity indices although larger multicentric trials need to be conducted.

Conclusion

There has been a need for a method, which can predict future development of PE by mid of pregnancy to choose one of the two lines of management—"To salvage or to terminate". Few authors have lately studied the use of serum angiogenic markers, and only a handful have shown its use in already established cases of early-onset preeclampsia.

This study is one of the few which establishes the use of serum PIGF biomarker assay in accurate prediction of early-onset preeclampsia as early as 20 weeks of gestation when the root cause can be "nipped in the bud." Significant low PIGF values are of prognostic value for rapid progression of maternal complication and fetal jeopardy, and therefore, further need for critical assessment and immediate management can be undertaken. Recent insight into the contribution of altered VEGF, sFLT-1 and PLGF concentrations in patients with preeclampsia may even introduce new therapeutic options.

Thus, we conclude that a woman with high risk factor who is screened positive by PIGF biomarker assay between 20 and 34 weeks of gestation has a 90.90 % chance of developing early-onset preeclampsia and a women screened negative will most certainly not develop it within next 4 weeks. An important aspect is the high negative predictive value for early-onset PE (100 %) which helps to detect those patients who will not develop early-onset PE, early positive prediction enabling one to take preventive measures sooner thus improving both maternal and perinatal prognosis.

We emphasize on the fact that PIGF biomarker assay is a novel study and a recent advance in reducing maternal and perinatal morbidity and mortality in early-onset preeclampsia and needs to be incorporated as a screening component in ANC profile of high-risk patients prone to develop early-onset preeclampsia.

Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical Approval The study was approved by the research ethics committee of the institution. All the procedures followed were in accordance with the ethical standards.

References

1. Menzies J, Magee LA, Macnab YC, et al. Current CHS and NHBPEP criteria for severe preeclampsia do not uniformly predict adverse maternal or perinatal outcomes. *Hypertens Pregnancy*. 2007;26:447–62.
2. Sibai BM. Diagnosis, controversies, and management of the syndrome of hemolysis, elevated liver enzymes, and low platelet count. *Obstet Gynecol*. 2004;103:981–91.
3. Magee LA, von Dadelszen P, Chan S, et al. The control of hypertension in pregnancy study pilot trial. *BJOG*. 2007;114:770.e13–20.
4. Chappell LC, Duckworth S, Seed PT, et al. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. *Circulation*. 2013. doi: [10.1161/CIRCULATIONAHA.113.003215](https://doi.org/10.1161/CIRCULATIONAHA.113.003215).
5. Maynard S, Min J, Merchan J, et al. Excess placental soluble FMS-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003;111:649–58.
6. Venkatesha A, Toporsian M, Lam C, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med*. 2006;12:642–9.
7. Levine R, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med*. 2006;355:992–1005.
8. Taylor R, Grimwood J, Taylor R, et al. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol*. 2003;188:177–82.
9. Buhimschi C, Norwitz E, Funai E, et al. Urinary angiogenic factors cluster hypertensive disorders and identify women with severe preeclampsia. *Am J Obstet Gynecol*. 2005;192:734–41.
10. Levine R, Maynard S, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004;350:672–82.
11. Wikstrom A, Larsson A, Eriksson U, et al. Placental growth factor and soluble fms-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. *Obstet Gynecol*. 2007;109:1368–74.
12. Romero R, Nien J, Espinoza J, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational neonate. *J Matern Fetal Neonatal Med*. 2008;21:9–23.
13. Ohkuchi A, Hirashima C, Matsubara S, et al. Alterations in placental growth factor levels before and after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. *Hypertens Res*. 2007;30:151–9.
14. Benton SJ, Hu Y, Xie F, et al. Angiogenic factors as diagnostic tests for preeclampsia: a performance comparison between two commercial immunoassays. *Am J Obstet Gynecol*. 2011;205:469.e1–8.
15. Sharon Maynard Annu. Rev. Med. 2008;59:61–78. Downloaded from arjournals.annualreviews.org by Mr Paul Sheard on 03/19/08.
16. Triage® PLGF Test Product Insert.2011.
17. Sibai BM. Hypertension. In: Gabbe SG, Niebyl JR, Galen H, et al., editors. *Obstetrics: Normal and problem pregnancies*. 6th ed. New York: Elsevier; 2012. p. 779–822.
18. Hirashima C, Ohkuchi A, Arai F, et al. Establishing reference values for both total soluble Fms-like tyrosine kinase 1 and free placental growth factor in pregnant women. *Hypertens Res*. 2005;28:727–32.
19. Cnossen JS, ter Riet G, Mol BW, et al. Are tests for predicting pre-eclampsia good enough to make screening viable? A review of reviews and critical appraisal. *Acta Obstet Gynecol Scand*. 2009;88:758–65.
20. Verloren S, Galindo A, Schlembach D, et al. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of preeclampsia. *Am J Obstet Gynecol*. 2009;201:e1–11.
21. Knudsen UB, Kronbrog CS, von Dadelszen P, et al. Pregnancy hypertension: an international Journal of Women's Cardiovascular. *Health*. 2012;2:8–15.