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Original Article

Biochemistry Section

Angiogenic Biomarker Placental Growth Factor (PLGF) in the Prediction and Diagnosis of Placental Dysfunction in Pre-eclampsia: A Cohort Study

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ABSTRACT

Introduction: Pre-eclampsia (PE), a pregnancy induced hypertensive disorder affects approximately 8-10% of all pregnancies in developing countries. A highly sensitive and specific marker for diagnosis of PE is the need of the hour, as diagnostic criteria are still based on non-specific clinical symptoms, ultrasound, and laboratory findings. Imbalance in the placental release of various angiogenesis regulatory factors to the maternal circulation is one of the significant contributors to its clinical manifestations. Low levels of pro-angiogenic biomarker Placental Growth Factor (PLGF) are detectable several weeks before clinical presentation of PE.

Aim: To determine the association of serum levels of PLGF with PE in second and third trimester of pregnancy.

Materials and Methods: A prospective cohort study was performed in the Department of Biochemistry, in collaboration with the Department of Obstetrics and Gynaecology at Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, from December 2018 to November 2021. A total of 130 patients were selected and divided into two groups, pre-eclamptic cases, normotensive controls. At enrolment in second trimester (24-28 weeks) and during third trimester (beyond 28 weeks) serum PLGF concentration was measured by using the Enzyme-linked Immunosorbent Assay (ELISA) kit method. Data was statistically analysed. Student's t-test was used for comparing differences between study groups. The Chi-square test was used to compare qualitative categorical data. Evaluation of the Area Under the Curve (AUC), diagnostic accuracy, sensitivity and specificity was done by Receiver Operating Characteristics (ROC) curve analysis done using software defined cut-off values.

Results: A total of 115 patients were analysed in the present study with 55 patients in group 1 (mean age: 25.83±3.27 years) and 60 patients in group 2 (mean age: 30.52±5.63 years) When compared with normotensive group PLGF levels were significantly lower in pre-eclamptic group with median 16.27 ng/mL versus 12.20 ng/mL ($p<0.001$) in second and 14.05 ng/mL versus 10.50 ng/mL ($p<0.001$) in third trimesters respectively. ROC curve analysis using cut-off point of 14.91 ng/mL showed sensitivity 80%, specificity 96.7%, AUC 0.896, 95% CI: (0.832-0.959) in second trimester and in third trimester at cut-off point of 13 ng/mL sensitivity 73%, specificity 96.7%, AUC 0.882 95% CI: (0.816-.948) was found.

Conclusion: PLGF may be used as a biomarker for early prediction, diagnosis, and management of PE. It might serve as ideal discriminating biochemical markers of PE. In the near future, the clinical utility of disease specific angiogenic biomarker in early detection of PE might improve health outcomes by preventing adverse maternal and neonatal outcomes and serious complications.

Keywords: Angiogenic imbalance, Diagnostic marker, Maternal mortality, Pregnancy induced hypertension, Soluble fms-like tyrosine kinase-1

INTRODUCTION

The PE, a hypertensive disorder of pregnancy affecting about 3-8% of pregnancies worldwide is a significant cause of maternal and perinatal morbidity [1]. A specific biomarker for diagnosis of PE is the need of the hour as diagnostic criteria are still based on non specific clinical symptoms, ultrasound and laboratory findings. It is mainly diagnosed by new onset of hypertension and proteinuria after 20 weeks of gestation or in the absence of proteinuria presence of one or more of the following: thrombocytopaenia, impaired liver functions, pulmonary oedema, renal insufficiency, or new onset of visual or cerebral problems [2]. These parameters are imprecise and have a low predictive value for disease progression and associated adverse outcomes. In India, out of 7.8% of total hypertensive disorders of pregnancy 5.6% is due to PE [3]. It can progress to multiorgan dysfunctions in the mother, including hepatic, cerebral, renal disease if the foetus and ischaemic placenta are not delivered [4,5]. It is mainly due to abnormal shallow invasion of trophoblast through decidua which alters remodeling of maternal spiral arteries leading to placental hypoxia [6]. Ischaemic placenta triggers release of various mediators including anti-angiogenic regulatory factors like soluble Feline McDonough Sarcoma (FMS) like tyrosine kinase (sFlt-1), endoglin and pro angiogenic factors like PLGF, Vascular Endothelial Growth Factor (VEGF) in maternal circulation causing endothelial dysfunction and associated complications of PE [7].

PLGF is a member of VEGF family along with VEGF A, B, C and D [8]. Members of VEGF family and its receptors play very important role in maintaining angiogenic homeostasis. PLGF is predominantly expressed in placenta mainly by trophoblasts and endothelial of human umbilical vein [9] and at low levels in the heart, lung, thyroid, liver, skeletal muscle and bone [8]. PLGF is pro-angiogenic as it enhances the activity of VEGF by competitively binding to the VEGF receptor VEGFR-1, allowing VEGF to bind then to VEGFR-2 which has stronger tyrosine kinase activity [10]. PE placenta produces higher concentration of anti-angiogenic biomarker soluble FMS-like tyrosine kinase-1 (sFlt-1), formed by alternate splicing of VEGFR-1. sFlt-1 when released into maternal circulation acts as decoy receptor to circulating VEGF and PLGF causing maternal endothelial dysfunctions and clinical manifestations of PE [11]. In the previous

part of the study, sFlt-1 data was analysed. In the present study, PLGF significance and association with PE is discussed. Separate kits were used to investigate both disease specific markers. They have independent significance for prediction and diagnosis of PE.

Angiogenic factors have emerged as important biomarkers in PE and the imbalance of these angiogenic markers is central to the pathogenesis of the maternal syndrome. Most of the complications of PE or related deliveries can be explained by alterations in angiogenic pathways [12]. Despite the understanding of these pathogenic processes is increasing, ability to manage these diseases has not improved accordingly as diagnostic criteria are still based on non-specific clinical, ultrasound and laboratory findings, rather than their pathogenic origin, and such diagnostic criteria have hardly been modified over the past decades [13-17].

In the quest of new biomarkers, the present cohort study was designed to determine circulating PLGF levels in second and third trimester of pregnancy to find its role as a biomarker in prediction, screening and diagnosis of PE by comparing with normotensive pregnant females. In India, pregnant women can also get benefit from this new diagnostic tool, as the reported prevalence of PE is on the higher side in India.

MATERIALS AND METHODS

A prospective cohort study was carried out in the Department of Biochemistry, in collaboration with Department of Obstetrics and Gynaecology in Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India. The study was done from December 2018 to November 2021 after taking approval by the Institutional Research and Ethical committee. (IEC clearance certificate no: Patho 903/18 dated 25.10.2018).

Inclusion criteria: A total of 130 study participants during second trimester of pregnancy (in 24-28 weeks) were enrolled in the present study and were selected by consecutive sampling from Obstetrics and Gynaecology Department.

Exclusion criteria: Patients with previous hypertension, renal diseases, cardiac diseases, diabetes mellitus, those with h/o smoking or drinking and those patients not willing to participate in the study were excluded.

Sample size calculation: The calculation was done using Epi Info software version 7.2.2.6 and was calculated using previous cohort study [13]. It was found to be 100 at 95% confidence interval and alpha error 5%. Considering drop-outs, 130 were taken as sample size.

Procedure

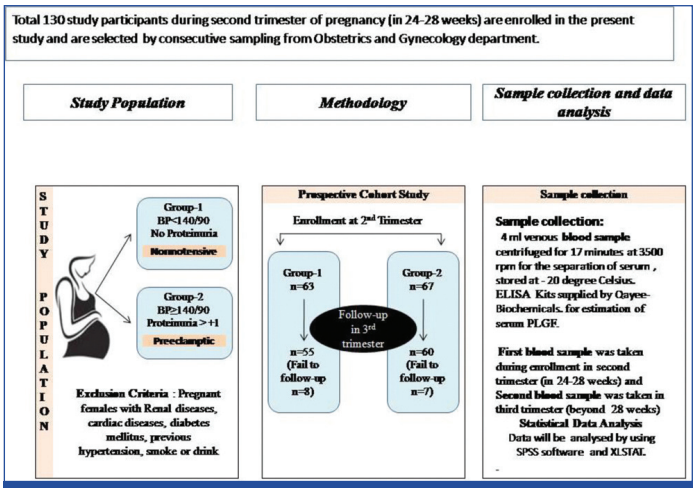
Before enrolment informed written consent was taken from all the study participants. Participants were subdivided into two groups after measuring Blood Pressure (BP) and proteinuria.

Group 1 (Normotensive controls): Pregnant women with normal BP and negative proteinuria or presence of urine proteins of +1 were enrolled in group1.

Group 2 (Pre-eclamptic cases): comprised of pregnant women with new onset of sustained elevated BP (≥140 mmHg systolic or ≥90 mmHg diastolic on at least two occasions around 6 hours apart) and proteinuria with presence of urine proteins of +2 or more occurring after 20 weeks of gestation or in the absence of proteinuria, new-onset hypertension with any one of the following: platelet count <100000/μL (thrombocytopaenia), renal insufficiency (serum creatinine concentration >1.1 mmg/dL), impaired liver functions with twice the normal concentrations of Aspartate Transaminase (AST) and Alanine Transaminase (ALT), pulmonary oedema, and new onset of visual or cerebral problems as per American College of Obstetricians and Gynaecologists task force classification on hypertension in pregnancy [14]. BP measurements were done by using calibrated sphygmomanometer in sitting position, after five minutes rest [14].

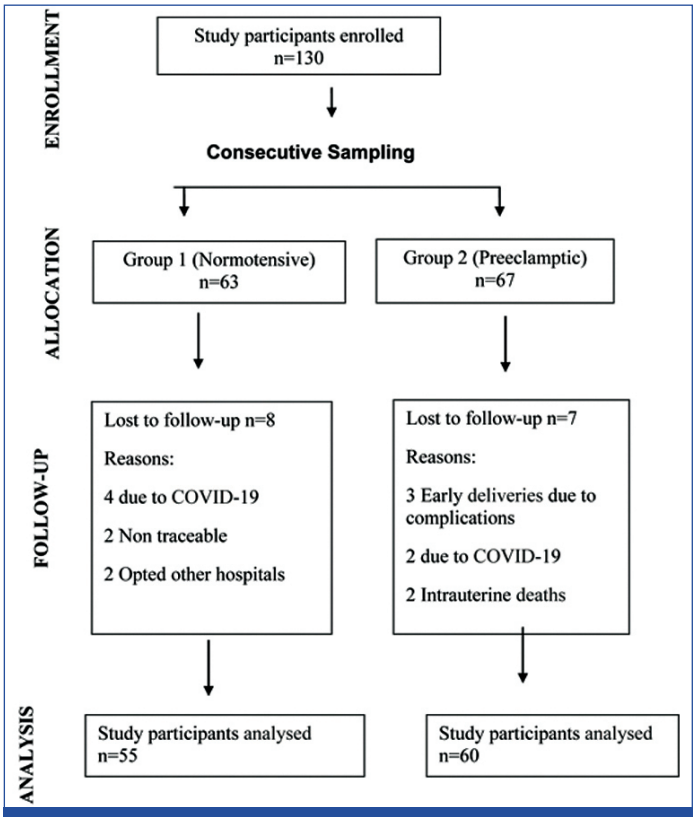
Semi-quantitative dipstick assay was done for urinary protein estimation. Using diagnostic reagent strips manufactured by SIEMENS Healthcare Private Limited. Data on demographic, general and obstetric characteristics was collected by using validated proforma including maternal age, height, weight, systolic BP and diastolic BP, gravidity and parity, history of PIH, family history of hypertension. All study participants of group1 and 2 were followed in third trimester (beyond 28 weeks).

A blood sample of 4 mL venous blood sample was taken from study participants under aseptic conditions and kept at room temperature for half an hour. Sample was centrifuged for 17 minutes at 3500 rpm for the separation of serum and was stored at-20ºC. Levels of serum PLGF were measured by the commercially available ELISA kit supplied by Qayee-Biochemicals with assay range: 6.25-200 ng/mL. First blood sample was taken during enrolment in second trimester (in 24-28 weeks) and second blood sample was taken in third trimester (beyond 28 weeks) for PLGF estimation [Table/Fig-1].



[Table/Fig-1]: Graphical representation showing the steps followed in the study.

[Table/Fig-2] represents flow chart of the study design, out of 63 study participants enrolled in group 1 and 67 study participants enrolled in group 2, eight participants in group1 and seven participants in group 2 were lost to follow-up.



[Table/Fig-2]: Flow chart of study design.

STATISTICAL ANALYSIS

For categorical data, analysis was done using percentage matrix and for demographic continuous data mean±standard Deviation (SD) was calculated. The Chi-square test was used to compare qualitative categorical data. Data was run through normalcy tests and was not found to be normally distributed. PLGF data was represented as median and interquartile range. Mann-Whitney U test and the Wilcoxon signed rank test were done to analyse PLGF data. ROC curve was applied to second and third trimester data for calculation of the AUC, software defined optimal cut-off values, sensitivity, specificity, lower bound and upper bound for 95% CI, Negative Predictive Value (NPV), Positive Predictive Value (PPV). p<0.001 was considered highly significant and p<0.05 was considered as significant for all the tests.

RESULTS

Demographic and obstetric characteristics of the study participants were analysed and significant differences in mean values of pre-eclamptic and normotensive group were seen as shown in [Table/Fig-3].

[Table/Fig-4] summarises the serum levels of PLGF concentrations (median and interquartile range IQR) in two groups in the second and third trimester. The concentrations were found to be lower in pre-eclamptic group in both the trimesters as compared with normotensive group. Second trimester median value in pre-eclamptic group was 12.20 ng/mL which was significantly less than the median values 16.27 ng/mL in normotensive group (p<0.001). Third trimester median value in normotensive group was 14.05 ng/mL as compared to10.50 ng/mL in pre-eclamptic group which was significantly different (p<0.001).

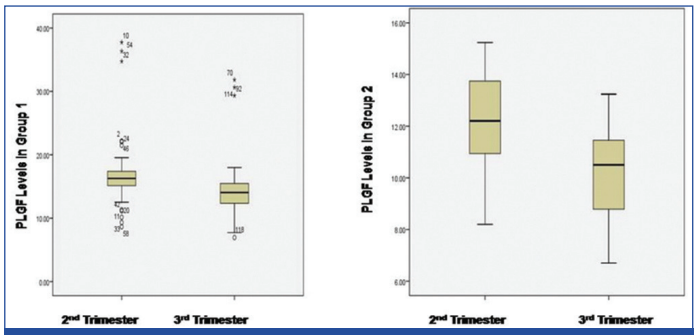
Box plot analysis was also performed for analysis of median values as shown in [Table/Fig-5a,b]. On ROC curve analysis, diagnostic accuracy 88.3%, sensitivity 80%, specificity 96.7%, PPV 96%, NPV 82% with AUC 0.896 at 14.91 ng/mL cut-off point (software defined) in second trimester and with diagnostic accuracy 85%, sensitivity 73%, specificity 96.7%, PPV 95.7%, NPV 78.4% with AUC 0.882 at cut-off point 13 ng/mL (software defined) in third trimester was observed [Table/Fig-6a,b].

S. No.	Characteristics	Normotensive (Group 1)	Pre-eclamptic (Group 2)	p-value
1.	Maternal Age(years)†	25.83±3.27	30.52±5.63	**<0.001****
2.	Maternal weight(kg)†	57.8±6.66	69.44±8.12	**<0.001****
3.	Maternal height (ft | in)†	5.34±.17	5.20±0.176	**<0.001****
4.	Systolic BP (mmHg)†	109.02±10.09	153.20±10.90	**<0.001****
5.	Diastolic BP (mmHg)†	72.36±7.019	100.63±5.13	**<0.001****
6.	Proteinuria‡	2 (3.6%)	55 (91.66%)	**<0.001****
7.	**Gravidity‡**			
	Primigravidae	10 (18.18%)	25 (41.6%)	**0.006**
	Multigravidae	45 (81.81%)	35 (58.3%)	
8.	**Parity‡**			
	Nulliparous	15 (27.27%)	33 (55 %)	**0.002****
	Primiparous	23 (41.81%)	18 (30%)	
	Multiparous	17 (30.9%)	9 (15%)	
9.	Previous history of PIH present‡	1 (1.66%)	34 (56.66%)	**<0.001****
10.	Family history of hypertension present‡	7 (11.66%)	16 (26.7%)	**0.014***

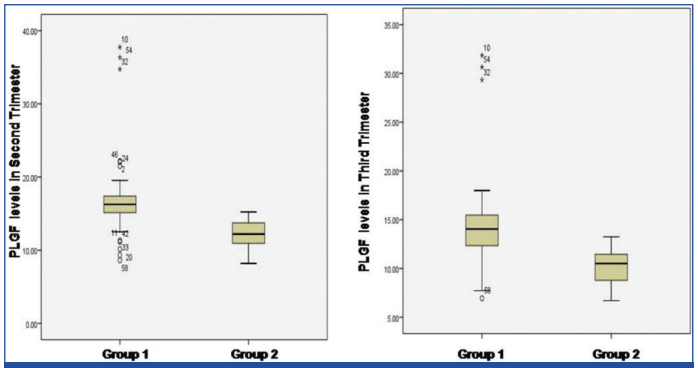
[Table/Fig-3]: PIH: Pregnancy Induced Hypertension.
†student (unpaired) t-test
‡The Chi-square test
p<0.05* statistically significant
p<0.001** statistically highly significant

Groups	PLGF (ng/mL)	Second trimester levels	Third trimester levels	p-value†
Normotensive group (Group 1)	Median	16.27	14.05	**p<0.001****
	IQR	15.10-17.45	12.32-15.48	
Preeclamptic group (Group 2)	Median	12.20	10.50	**p<0.001****
	IQR	10.895-13.74	8.767-11.50	
p-value‡		**p<0.001****	**p<0.001****	

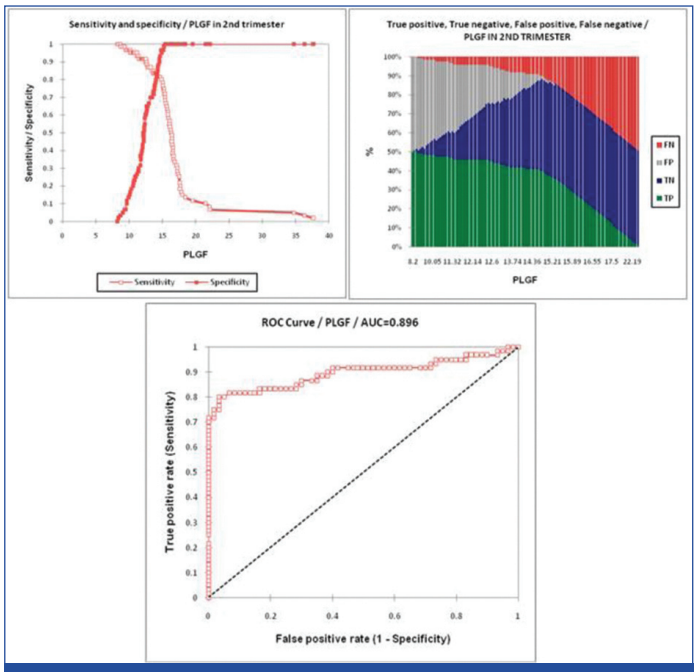
[Table/Fig-4]: Comparison of PLGF (ng/mL) levels in second and third trimester of normotensive group and pre-eclamptic group.
†Wilcoxon signed rank test
‡Mann-Whitney U test
p<0.05* statistically significant
p<0.001** statistically highly significant



[Table/Fig-5a]: Represents box plots showing serum concentrations of PLGF in group 1 (normotensive group) and group 2 (pre-eclamptic group).



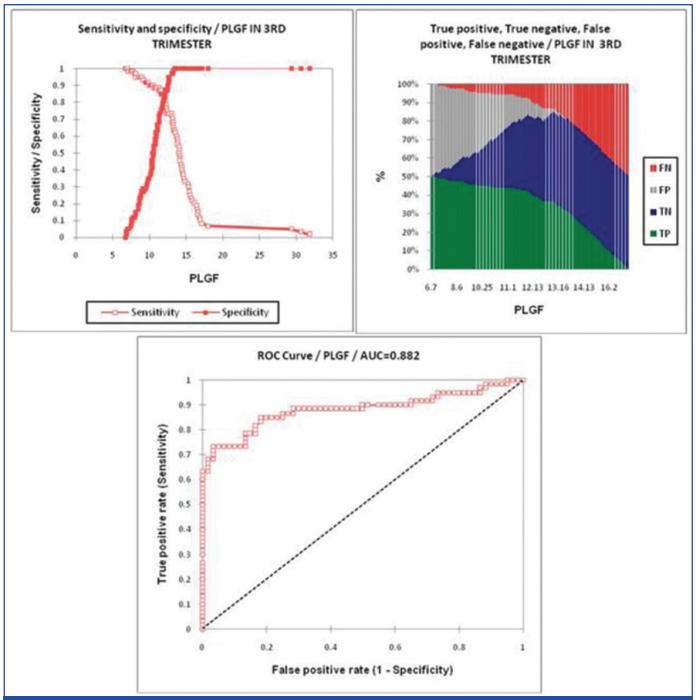
[Table/Fig-5b]: Representing box plots showing comparison of levels in second and third trimesters of group 1 and group 2.



[Table/Fig-6a]: Represents ROC curve, AUC and predictive characteristics for estimation of cut-off points of PLGF levels in second trimester.

DISCUSSION

The PE is a multifactorial disorder of pregnancy and one of the main causes of maternal and neonatal mortality and morbidity in the world [12]. The pathophysiology of PE have highlighted a role of



[Table/Fig-6b]: Shows ROC curve, AUC and predictive characteristics for estimation of cut-off points of PLGF levels in third trimester.

placentally derived angiogenic factors as relevant disease biomarker [13,18-20]. The classical diagnostic clinical markers of hypertension and proteinuria are imprecise and are of poor predictive value for disease progression and associated adverse outcomes [15,16]. Timely and accurate diagnosis of PE is still a major challenge [17].

It was seen that a family history of hypertension, previous history of Pregnancy Induced Hypertension (PIH), Advanced Maternal Age (AMA), nulliparity, overweight, proteinuria, systolic and diastolic BP were significantly associated with PE when compared with normotensive pregnant females whereas gravidity was not significantly associated with PE. These findings were consistent with various other previous studies [19,21]. Various recent studies found that extremes of childbearing age are linked with risk of PE/eclampsia. Risk of PE is almost twice in women of AMA. Nulliparity and family history of PE almost triple the risk of PE. Body Mass Index (BMI) is considered a strong risk factor as PE risk increases with a greater BMI even within the normal range. The worldwide increase in obesity causes an increase in the frequency of PE and is one of the largest attributable and modifiable risk factors for PE [5,21].

Results of the study were consistent with previous prospective cohort studies, showing PLGF levels to be lower in pre-eclamptic group in both trimesters [13,22]. Significant difference in median PLGF levels ($p<0.001$) both in second and third trimester of pre-eclamptic and control group was observed with median (200 vs 961.5 pg/mL) in second trimester and median (90.7 vs 852 pg/mL) in third trimester in study done by De Vivo A et al., [22]. In the study done by Radulescu C et al., levels were significantly lower in PE with median values (319.31 vs 387.07 pg/mL) in second trimester and (313.36 vs 696.93 pg/mL) in third trimester of pre-eclamptic and control group respectively [13]. A study done by McElrath TF et al., observed that highest median concentration of PLGF were found in women with uncomplicated normotensive pregnancies approximately from 17 weeks of gestation, intermediate concentrations were found in women with gestational hypertension and lowest in those with PE [23].

These findings can be due to proposed hypothesis that decrease in circulatory PLGF concentrations in PE are largely due to excessively produced VEGF receptor sFlt-1 that binds PLGF leading to decrease in free or unbound levels of PLGF [24]. Due to hypoxia induced alternative splicing of Flt-1RNA, expression of antiangiogenic factor sFlt-1 is upregulated in PE. sFlt-1 when released into maternal circulation act as decoy receptor to circulating VEGF and PLGF. These alterations are reflected in the form of angiogenic imbalance with increase in sFlt-1/PLGF ratio which leads to maternal endothelial dysfunctions, pathological changes and clinical manifestations of PE [5,7].

With reference to AUC obtained during ROC curve analysis, the current study suggests that PLGF can be used as early predictive marker of PE with diagnostic accuracy 88.3%, sensitivity 80%, specificity 96.7 %, PPV 96%, NPV 82% with AUC 0.896 at 14.91 ng/mL cut-off point in second trimester and with diagnostic accuracy 85%, sensitivity 73%, specificity 96.7%, PPV 95.7%, NPV 78.4% with AUC 0.882 at cut-off point 13 ng/mL in third trimester. A systematic review and meta-analysis done by Agarwal S et al., also concluded that PLGF is a useful screening tool to predict PE [25]. However, this review which includes various studies exploring PLGF as a predictor of PE showed a wide variation in diagnostic accuracy of PLGF ranging between 45% and 95%. It can be explained by the mixed patient population comprising study groups, varied software defined cut-off values used due to different analysis platforms, lack of distinction between early and late onset PE [25]. A study done by Tsiakkas A et al., demonstrates that during second and third trimesters of PE, addition of serum PLGF improves the prediction and performance of screening provided by maternal factors alone [26]. A recent stratified analysis done by Duhig KE et al., revealed that PLGF testing decreases time to recognise PE and may reduce associated severe maternal adverse outcome [20].

Significance of altered PLGF in PE was reviewed in a recent study done by Stepan H et al., who proposed to redefine the American College of Obstetrics and Gynecologists (ACOG) definition in the near future by including new onset of altered angiogenic markers (PLGF alone or sFlt-1/PLGF ratio) along with newly diagnosed hypertension. This study also suggested that PE term should be evolved to angiogenic-placental syndrome reflecting placenta generated imbalance in angiogenic state leading to clinical manifestations of disease [19]. Use of angiogenic biomarker PLGF might help in screening and faster diagnosis of pre-eclamptic patients and also prevent them from adverse maternal and neonatal outcomes and serious complications.

Limitation(s)

The main limitation of the present study was that the adverse outcomes and associated complications were not observed as all study participants could not be followed till delivery due to Coronavirus Disease-2019 (COVID-19).

CONCLUSION(S)

In the current study, significantly lower PLGF levels were found in second and third trimesters of PE when compared with normotensive pregnancy. The current study suggests that disease specific biomarker PLGF can be used as sensitive laboratory test and ideal biochemical marker for screening, early detection and diagnosis of PE. It can also improve health outcomes by providing better information about state of disease. However, future research is recommended in Indian set-up to validate the clinical applications of these biomarkers.

Declaration

The authors want to declare that this study was a part of the PhD Medical Biochemistry Research project titled “Association of angiogenesis related biomarkers PLGF, sFlt-1 and sFlt-1/PLGF with PE in second and third trimester of pregnancy” done from 2018 to 2021; a part of which has been previously published titled “Antiangiogenic Biomarker Soluble Fms-like Tyrosine Kinase-1 in Pregnancy Complicated with PE”.

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