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Original Article

Biochemistry Section

Antiangiogenic Biomarker Soluble Fms-like Tyrosine Kinase-1 in Pregnancy Complicated with Preeclampsia: A Cohort Study

MANDEEP KAUR[1], SANGEETA PAHWA[2], ROHIT ARORA[3], NAMRATA CHHABRA[4], JASNEET KAUR[5], SAHIBA KUKREJA[6]

ABSTRACT

Introduction: Preeclampsia (PE) is one of the leading obstetric diseases with significant morbidity and mortality in both mother and foetus. The etiology of preeclampsia is unknown. It may result from several reasons with imbalance between angiogenic regulatory factors in maternal circulation as one of the factor. High circulatory levels of Soluble Fms-like Tyrosine Kinase-1 (sFlt-1) are detectable several weeks before clinical presentation of preeclampsia.

Aim: To determine association of serum levels of sFlt-1 with preeclampsia in second and third trimester of pregnancy.

Materials and Methods: A prospective cohort study was conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynaecology in a tertiary care hospital (Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India), from November 2018 to March 2021. The study participants were divided into two groups i.e, normotensive (group 1) and preeclamptic (group 2). The enrollment of participants was done during second trimester. Serum sFlt-1 concentration was measured in second trimester (24-28 weeks) and in third trimester (beyond 28 weeks) using Enzyme Linked Immunoassay (ELISA) kits. Receiver Operating Characteristics (ROC) curve analysis was done for evaluation of Area Under Curve (AUC), sensitivity and specificity using software defined cut-off values.

Results: Total 60 participants were there in each group with maternal mean age of 25.8±3.2 years in group 1 and 30.6±5.5 years in group 2 (p-value <0.001). sFlt-1 levels were significantly higher in preeclampsia group both in second and third trimesters when compared with normotensive group with median 313.07 versus 65.150 (p-value <0.001) and 337.875 versus 76.925 (p-value <0.001), respectively. The ROC curve analysis using 190.5 ng/mL as cut-off point in second trimester showed sensitivity 90%, specificity 85%, AUC was 0.832, 95% CI (0.745-0.918) and in third trimester at cut-off point 271.5 ng/mL showed sensitivity 90%, specificity 90%, AUC was 0.884, 95% CI (0.817-0.951).

Conclusion: The soluble Fms-Like Tyrosine Kinase-1-1 (sFlt-1), may serve as biomarker for early diagnosis and can improve prediction of preeclampsia.

Keywords: Angiogenic imbalance, Clinical manifestations, Diagnosis, Early prediction, Second and third trimester

INTRODUCTION

Preeclampsia (PE) is one of life threatening complication of pregnancy affecting 3-8% of total pregnancies worldwide, leading to about 60,000 maternal and 500,000 perinatal deaths per year [1]. In India, prevalence of hypertensive disorders of pregnancy is 7.8% and of preeclampsia is 5.6% [2]. Preeclampsia is presented with new onset of hypertension and proteinuria in mother during second trimester after 20 weeks, or in the absence of proteinuria with co-existence of one or more of the following: thrombocytopenia, renal insufficiency, impaired liver functions, pulmonary oedema, or new onset of visual or cerebral problems [3]. The pathogenic process begins during first trimester, much before the clinical signs appear [4]. The causes and underlying pathophysiology are still uncertain, however its pathology is considered under two stages [4,5]. First stage is preclinical and is due to defects in the trophoblastic vascular remodelling of spiral arteries of uterus leading ultimately to placental hypoxia and ischemia [5,6]. Second stage is of maternal syndrome which is due to release of various placental factors into maternal circulation causing inflammatory response, endothelial dysfunctions, and long term complications [7]. Angiogenic regulatory factors are one of the important placental factors released in maternal circulation during preeclampsia.

Various proangiogenic factors like Placental Growth Factor (PLGF), Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor-β (TGF-β) and antiangiogenic factors like VEGF receptor (VEGFR-1/Flt-1), soluble VEGFR-1 (sFlt-1), soluble endoglin (sENG) are considered to be effective in early prediction, diagnosis and management of the disease [8,9]. Various recent studies revealed that expression of placental antiangiogenic factors Flt-1 is the major contributor for the clinical manifestations of preeclampsia [3,10].

Hypoxic and ischaemic placenta produces higher concentration of soluble fms-like tyrosine kinase-1, a truncated and spliced form of VEGFR-1. It is formed by alternate splicing of Flt-1 and contains only first 6 out of seven immunoglobulin like extra cellular domains and lack intracellular signaling domain and transmembrane domains [11,12]. sFlt-1 plays a vital role in angiogenesis and vasculogenesis.

The present prospective cohort study was designed to determine circulating sFlt-1 levels in second and third trimester of pregnancy to find association of antiangiogenic factor sFlt-1 as a biomarker in screening and diagnosis of preeclampsia.

MATERIALS AND METHODS

A prospective cohort study was conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynaecology in a tertiary care hospital (Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India), from November 2018 to March 2021. The ethical approval was taken by the Research and Ethical Committee (No: Patho 903/18 dated 25.10.2018).

Inclusion and Exclusion criteria: A total of 140 study participants during second trimester of pregnancy (in 24-28 weeks) were included in the present study. Women who had chronic medical conditions such as renal diseases, cardiac diseases, diabetes mellitus, previous hypertension, smoke or drink were excluded from the study group.

Sample size calculation: Sample size was calculated using Epi Info software version 7.2.2.6 using previous study [2] and found to be 100 at 97% confidence interval and alpha error 5%. Considering dropouts, 140 were calculated as sample size.

Study participants were selected by non randomisation from Department of Obstetrics and Gynaecology. Informed written consent was taken from all the study participants. After measuring blood pressure and proteinuria participants were categorised into two groups.

- Group 1 (Normotensive) consisted of pregnant women with normal Blood Pressure (BP) and negative proteinuria or presence of urine proteins of +1.
- Group 2 (Preeclamptic) consisted of pregnant women with newly diagnosed hypertension (systolic BP of 140 mmHg or more and/or diastolic BP of 90 mm Hg or more) on two occasions at least 4 hours apart and proteinuria with presence of urine proteins of +2 or more after 20 weeks of gestation.

Blood pressure was measured in sitting position after 5 minutes rest using calibrated sphygmomanometer. Urinary protein estimation was done by semi quantitative dipstick assay.

Validated structured performa was used to collect data on demographic, Obstetric characteristics, previous history of patient, and family history. All study participants of group 1 and 2 were followed-up in third trimester (beyond 28 weeks).

Procedure

Total 4 mL of venous blood sample was taken in red top vacutainer from antecubital vein of study participants and kept at room temperature for half an hour. It was centrifuged for 17 minutes at 3500 rpm for the separation of serum and was stored at -20° Celsius until use. Levels of serum sFlt-1 were measured by the commercially available kit supplied by Qayee-Biochemicals which is based on double-antibody sandwich Enzyme-Linked Immunosorbent (ELISA) One-Step Process Assay using assay range: 12.5 ng/mL-800 ng/mL.

First blood sample was taken during second trimester (in 24-28 weeks) while enrollment in the study and the second blood sample was taken in third trimester (beyond 28 weeks) for sFlt-1 estimation.

STATISTICAL ANALYSIS

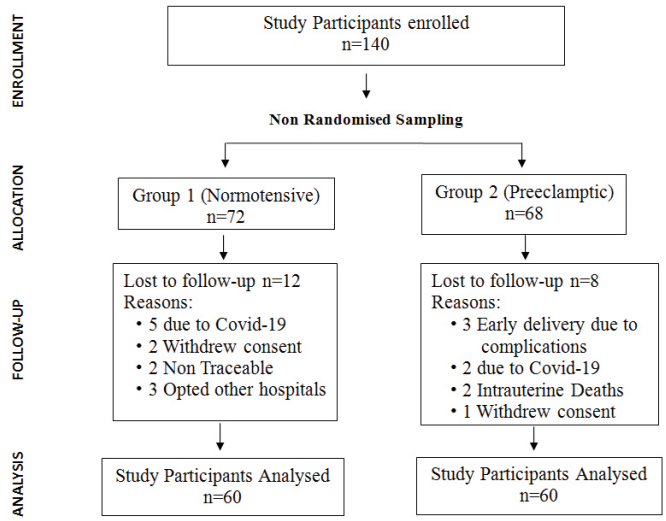
Data was represented as percentage for categorical data, mean±Standard Deviation (SD) for demographic continuous data, and median and Interquartile Range (IQR) for levels of sFlt-1. Wilcoxon signed rank test and the Mann-Whitney U test were done to analyse the data. Receiver Operating Characteristics (ROC) curve was used to find Area Under Curve (AUC) and optimal cut-off values, lower bound and upper bound for 95% confidence interval, calculation of Negative Predictive Values (NPVs), positive predictive values (PPVs), sensitivity and specificity of the test. A p-value <0.05 was considered as significant, while p-value <0.001 was considered highly significant for all the tests.

RESULTS

Out of 140 pregnant women enrolled, 12 participants in group 1 and eight participants in group 2 were lost to follow-up [Table/Fig-1].

Demographic and obstetric characteristics of preeclamptic and normotensive group were analysed and significant difference in mean values was seen as shown in [Table/Fig-2].

[Table/Fig-3] summarises the serum levels of sFlt-1 in two groups in the second and third trimester. The concentrations



[Table/Fig-1]: Flow chart of study design.

Charateristics	Group 1	Group 2	p-value
Maternal age (years)	25.8±3.2	30.6±5.5	<0.001
Maternal weight (kg)	58.2±7.0	69.2±7.2	<0.001
Maternal height (cm)	5.3±.17	5.2±0.179	<0.001
Body mass index (kg/m^2)	21.88±3.07	27±2.8	<0.001
Systolic blood pressure (mmHg)	108.83±9.75	153.17±11.01	<0.001
Diastolic blood pressure (mmHg)	72.83±7.15	90.58±6.9	<0.001
Proteinuria	2 (3.3%)	55 (91.7%)	<0.001
Gravidity			
Primagravida	12 (20%)	26 (43.3%)	0.006
Multigravida	48 (80%)	34 (56.7%)	
Parity			
Nulliparous	16 (26.7%)	34 (56.7%)	0.002
Primiparous	24 (40%)	18 (30%)	
Multiparous	20 (33.3%)	8 (13.3%)	
Previous history of pregnancy induced hypertension present	0	30 (50%)	<0.001
Family history of hypertension present	6 (10%)	16 (26.7%)	0.018

[Table/Fig-2]: Demographic and obstetric characteristics of the study population.

were represented as median and Interquartile Range (IQR). Levels were found to be higher in the preeclamptic group in both the trimesters as compared with normotensive group. Second trimester median value in normotensive group was 65.150 which was significantly less than the median values 313.07 in preeclamptic group (p-value <0.001). Third trimester median value in normotensive group was 76.925 as compared to 337.875 in preeclamptic group which was significantly different (p-value <0.001).

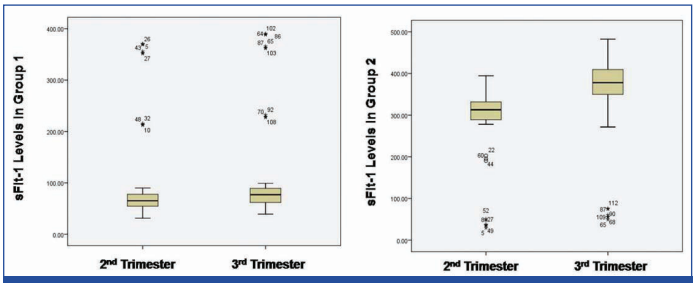
Groups		Second trimester sFlt-1 (ng/mL) levels	Third trimester sFlt-1 (ng/mL) levels	p-value
Group 1	Median	65.150	76.925	<0.001
	Interquartile range	54.46-78.47	61.375-89.415	
Group 2	Median	313.07	337.875	<0.001
	Interquartile range	288.287-331.825	348.725-412.700	
p-value		<0.001	<0.001	

[Table/Fig-3]: Comparison of sFlt-1 (ng/mL) levels in normotensive and preeclamptic group.

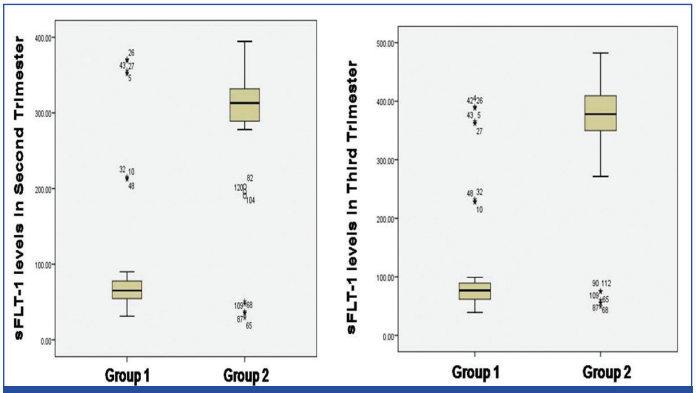
Box plot analysis was also performed for analysis of values as shown in [Table/Fig-4a,b].

On ROC curve analysis, in second trimester diagnostic accuracy 87.5%, sensitivity 90%, specificity 85%, PPV 85.7%, NPV 89.5%

with AUC was 0.832 at 190.5 ng/mL cut-off point, 95% CI (0.745-0.918) was observed, while in third trimester the diagnostic accuracy 90%, sensitivity 90%, specificity 90%, PPV 90%, NPV 90% with AUC 0.884 at cut-off point 271.5 ng/mL, 95% CI (0.817-0.951) was observed.

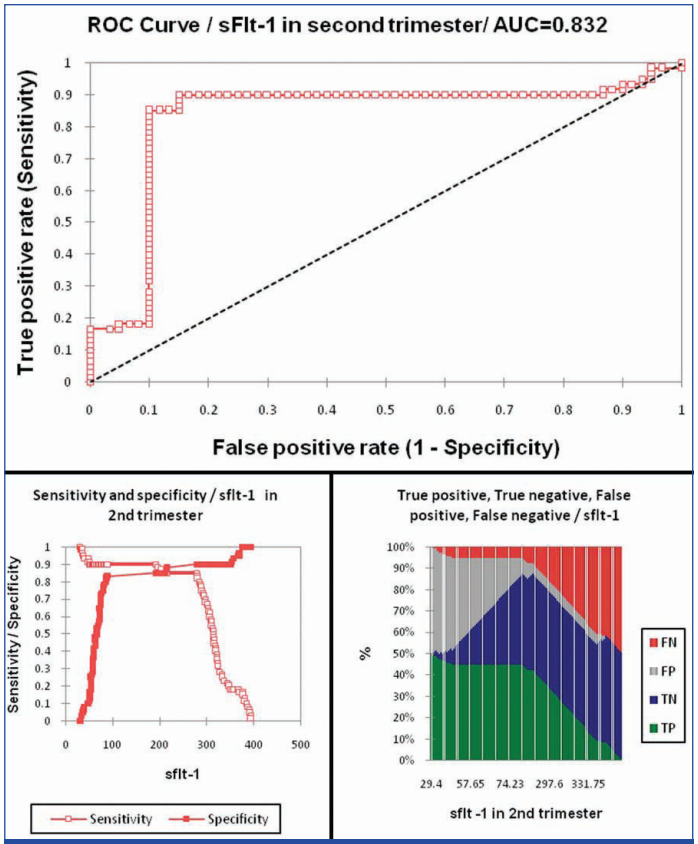


[Table/Fig-4a]: Box plot showing serum concentrations of sFlt-1 in normotensive and preeclamptic group.

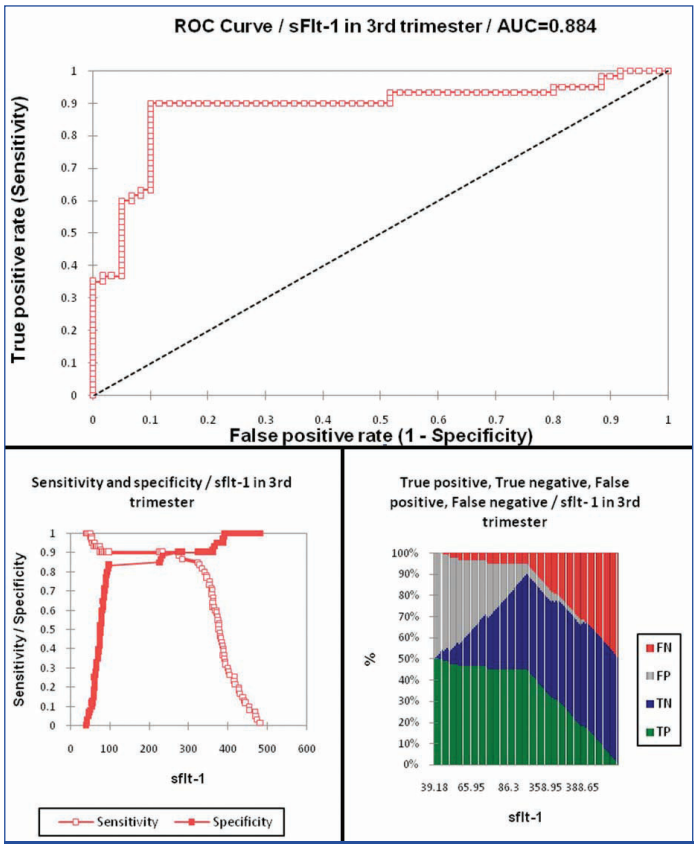


[Table/Fig-4b]: Box plot showing comparison of levels in second and third trimesters of normotensive and preeclamptic group.

With reference to AUC obtained during ROC curve analysis, the present study suggested that sFlt-1 can be used as early predictive marker of preeclampsia with diagnostic accuracy 87.5%, sensitivity 90%, specificity 85%, PPV 85.7%, NPV 89.5% with AUC was 0.832 at 190.5 ng/mL cut-off point in second trimester [Table/Fig-5a] and with diagnostic accuracy 90%, sensitivity 90%, specificity 90%, PPV 90%, NPV 90% with AUC was 0.884 at cut-off point 271.5 ng/mL in third trimester [Table/Fig-5b].



[Table/Fig-5a]: Shows ROC curve for estimation of cut-off points of sFlt-1 levels in second trimester.



[Table/Fig-5b]: Shows ROC curve for estimation of cut-off points of sFlt-1 levels in third trimester.

DISCUSSION

Preeclampsia is a multi-system disorder for which clinical criteria alone are insufficient to predict adverse outcomes [9]. Till date, the only effective treatment of preeclampsia is early delivery of the foetus and ischaemic placenta [11,14]. Reliable and early prediction of PE and related clinical maternal and foetal adverse outcomes is the need of the hour in antenatal care [15, 16]. An angiogenic imbalance, resulting from an inappropriately elevated increase in sFlt-1 expression is responsible for progression of the maternal syndrome of preeclampsia [17]. High levels of sFlt-1 are seen in women on high risk of developing PE, significant increase usually detected 5-6 weeks before the onset of clinical presentation of disease [4].

In the present study as depicted in [Table/Fig-2], it was observed that maternal age, nulliparity, family history of hypertension, previous history of Pregnancy Induced Hypertension (PIH) and overweight were significantly higher in group 2 as compared to group 1. Various recent published studies also found higher incidence of preeclampsia among older women. Women of Advanced Maternal Age (AMA) were two times more likely to have preeclampsia [14]. This study also conclude that nulliparity is a strong risk factor which almost triples the risk of preeclampsia (odds ratio 2.91, 95% confidence interval (1.28-6.61). Preeclampsia is believed as a disease of first pregnancy and the risk of preeclampsia is increased nearly three times in patients with family history of preeclampsia. Body Mass Index (BMI) is also considered an associated factor as risk of preeclampsia increases progressively with increasing BMI even within the normal range. Overall risk of preeclampsia increases approximately twofold to threefold in obese pregnant women [14,18].

A significant increase of sFlt-1 in preeclamptic group both in second (p-value <0.001) and third (p-value <0.001) trimester was observed. In the current study as shown in [Table/Fig-3] second and third trimester median values in normotensive group were 65.150 (IQR=54.46-78.47) and 76.925 (IQR=61.375-89.415), respectively which was significantly less than the median values 313.07 (IQR=288.287-331.825) and 337.875 (IQR=348.725-412.700) in preeclamptic group. Results of the study were in accordance with the literature showing sFlt-1 to be higher in preeclamptic group in both trimesters [19,20]. A cohort study done by Rădulescu C et al., also showed significant difference in median sFlt-1 levels both in second and third trimester, in preeclamptic and control group with median (310.22 vs 142.05) in second and (514.23 vs 201.32) in third trimester respectively [19]. Abbas AM et al., in their observational study showed that mean sFlt-1 value in the control group was 53.9±24.6 which was significantly less than the sFlt-1 value in severe preeclamptic group (96.2±31.1, p<0.001) and eclampsia groups (213.2±104.3, p<0.001) [9]. Complicated preeclamptic group showed higher sFlt-1 levels than in non-complicated cases (120.2±19.6 versus 72.2±19.6, p-value <0.001). The present study findings were also consistent with data of other previous observational studies showing angiogenic imbalance due to excessive release of sFlt-1into maternal circulation [11,21,22]. Various recent studies have also confirmed that in the triage setting along with PLGF levels, sFlt1 can be used as a robust prognostic test to ensure appropriate treatment and it also helps in reducing unnecessary hospitalisation of suspected pre-eclamptic cases [15,23].

The above findings could be due to hypoxia induced alternative splicing of Flt-1 RNA which upregulates expression of antiangiogenic factor sFlt-1. The sFlt-1 is further released into maternal circulation and act as decoy receptor to circulating VEGF and PLGF. sFlt-1 antagonises ligand mediated angiogenic signalling through cell surface receptors and causes angiogenic imbalance by inhibiting the beneficial effects of proangiogenic factors on maternal endothelium, thus leading to maternal endothelial dysfunctions and clinical manifestations of preeclampsia [1,13,24]. During screening of preeclampsia by serum sFlt-1, diagnosis in early third trimester could be improved by inclusion of measurements from second trimester. This was also stated by previous study done by Wright D et al., [21].

In the study done by Abbas AM et al., during ROC analysis sensitivity 90% and specificity 80% with AUC was 0.923 at cut-off point 102.60 ng/mL was observed [9]. Significant association between sFlt-1 and maternal complications in preeclampsia was also reported in this study.

Limitation(s)

The main limitation of the present study was that the participants could not be followed till delivery to see the adverse outcomes and associated complications due to COVID-19. Research is still required in the Indian set-up to validate the results.

CONCLUSION(S)

The present study data taken together with those of previous studies showed that serum anti-angiogenic factor sFlt-1 level detection in second and third trimester may help in early prediction, diagnosis and management by providing better information about state of disease as the levels were significantly higher in preeclampsia than in normal pregnancy. It might be used as sensitive laboratory test for screening preeclampsia.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
2. Professor and Head, Department of Obstetrics and Gynaecology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
3. Assistant Professor, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
4. Professor and Head, Department of Biochemistry, Sir Seewoosagur Ramgoolam Medical College Belle-Rive, Vacoas-Phoenix, Mauritius.
5. Intern, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
6. Professor and Head, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Mandeep Kaur,
81/696-A, Guru Nanak Avenue, Phase 3, Backside Power Colony,
Majitha Road, Amritsar-143001, Punjab, India.
E-mail: mandeep2974@gmail.com

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