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Role of soluble fms-like tyrosine kinase-1/placental growth factor ratio along with uterine artery Doppler for the prediction of pre-eclampsia – A case–control study

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ABSTRACT

Background: Hypertensive disorders of pregnancy are becoming the leading cause of maternal morbidity and mortality worldwide and are responsible for 9–25% of deaths 1. They are believed to occur due to an imbalance between pro-angiogenic factors, like placental growth factor (PlGF) and anti-angiogenic factors, like soluble fms-like tyrosine kinase-1 (sFLT-1). **Aims and Objectives:** The aim of the study was to evaluate the role of the sFLT-1/PlGF ratio along with uterine artery Doppler for the prediction of pre-eclampsia (PE). **Materials and Methods:** The current study was a prospective case–control study conducted at Sri Ramachandra Medical College and Hospital, Chennai, from 2018 to 2020. Blood samples for sFLT-1 and PlGF and uterine artery Doppler were done in 100 pregnant mothers who are at 16–20-week gestation attending antenatal outpatient department in the Department of Obstetrics and Gynecology. **Results:** We found that the mean sFLT-1 in high-risk group was 826.17 ng/L (standard deviation [SD] ± 251.31) compared to 924.69 ng/L (SD ± 360.61) in low-risk groups. The mean PlGF in the high-risk group was 23.07 ng/L (SD ± 4.68) compared to 27.43 ng/L (SD ± 5.62) in low-risk group. The mean sFLT-1/PlGF ratio was increased in high-risk group of about 39.68 (SD ± 22.77) compared to 35 (SD ± 16.98) in low-risk group. Women with high resistance uterine artery Doppler have 8.5 odds of getting PE compared to those with normal uterine artery Doppler. **Conclusion:** According to our study, the sFLT-1/PlGF ratio carries more sensitivity (90%) and negative predictive value (74.19%) if we keep the value as 32.25 along with uterine Doppler rather than their individual values for the prediction of PE.

Key words: Soluble fms-like tyrosine kinase-1; Placental growth factor; Uterine artery Doppler; Pre-eclampsia

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INTRODUCTION

Pre-eclampsia (PE) is described as a pregnancy-specific multi-system disorder characterized by hypertension (HTN) (≥140/90) and proteinuria after 20 weeks of gestation. It is associated with significant maternal and perinatal morbidity and mortality in the developing countries.

The etiopathogenesis of PE is not completely known but recent studies have demonstrated that this disease appears to originate in the placenta and is characterized by abnormal trophoblastic invasion and failure of remodeling of the spiral arterioles leads to a high resistance uteroplacental circulation that can be detected by uterine artery Doppler in ultrasound.[1] Various studies have revealed that an imbalance between angiogenic factors, such as placental growth factor (PlGF) and anti-angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFLT-1) is related to the pathogenesis of PE.[2-10]

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Till date, termination of the pregnancy is the definite treatment for PE. Several attempts have been made to identify early markers of defective placentation, impaired placental circulation, and endothelial dysfunction that may help in predicting women who are likely to develop PE. Maternal serum concentrations of these biomarkers either increase or decrease in PE during gestation. This study focuses on the role of SFLT-1, PlGF, and their ratio along with uterine artery pulsatility index in early gestation in predicting early- and late-onset PE.

Aims and objectives

The aim of the study was to evaluate the role of sFLT-1/PlGF ratio along with uterine artery Doppler for the prediction of PE.

MATERIALS AND METHODS

Source of data

The study was conducted in the Department of Obstetrics and Gynecology and Fetal Medicine Unit for uterine artery Doppler velocimetry in SRMC and RI, Porur, Chennai, for 12–15 months. 100 pregnant mothers with gestational age between 16 and 20 weeks attending the antenatal outpatient department in the Department of Obstetrics and Gynecology were included in this study.

Inclusion criteria

The following criteria were included in the study:

- Singleton pregnancy
- History of hypertensive disorders of pregnancy (HDP) such as gestational HTN (GHTN), PE, eclampsia, HELLP syndrome in previous pregnancies
- Elderly women
- Family history of PE (for example, PE in mother or sister)
- Pre-pregnancy body mass index (BMI) ≥35
- Clinical history suggestive of antiphospholipid antibodies (APLA)
- Conceived after artificial reproductive techniques
- APLA positive.

Exclusion criteria include

The following criteria were excluded from the study:

- Multiple pregnancies
- Molar pregnancy
- Chronic HTN
- Pregestational diabetes mellitus
- Connective tissue disorders
- Any liver/kidney diseases
- Any bleeding disorder.

Women who fulfilled the inclusion criteria (70 of them) were taken in the study group. Thirty of them with no risk factors were allocated to the control group (nulliparous, normal BMI, singleton, spontaneous conception, no family history of or previous history of PE or eclampsia).

Parameters used were age, obstetric score, BMI, blood pressure, blood serum samples for sFLT-1 and PlGF, TVS pelvis for mean uterine artery Doppler pulsatility index (PI). All the data collected in the proforma were compiled and statistical analysis was done using the SPSS 4.0 version.

Ethics Committee approval obtained from the institute–REF: IEC-NI/18/JAN/63/11.

Methodology

Detailed maternal history such as age, parity, gestational age, onset of symptoms, and associated risk factors was taken followed by clinical examination including BP measurement in a sitting or left lateral position. After getting informed written consent, apart from routine antenatal investigations, blood samples for SFLT-1 and PlGF were taken from all the 100 pregnant mothers followed by uterine artery Doppler PI by transvaginal ultrasound was done at 16–20 weeks of gestational age. Blood samples were stored in a cold freezer till processing. Patients were followed up till delivery for the development of PE and its complications.

Study duration

6–9 months for sample collection+6-month follow-up (2018–2020).

Study design

Prospective case–control study.

Study population

100 pregnant women–70 study group/30 control group.

Parameters used

Age, obstetric score, BMI, blood pressure, blood serum samples for sFLT-1 and PlGF, TVS pelvis for mean uterine artery Doppler PI.

Statistics

All the data collected in the proforma were compiled and statistical analysis was done using the SPSS 4.0 version.

RESULTS

All the data collected in the pro forma were taken for results and were evaluated with appropriate statistical analysis.

Among 100 women who were examined in this study, 30 women in the control group were considered as low risk

and 70 women in the study group were considered as high-risk cases.

Demographic characteristics of the study population		
Characteristics	**Risk (%)**	
	High-risk group	**Low-risk group**
Gravida		
Primi	38 (54.3)	20 (66.7)
Multi	32 (45.7)	10 (33.3)
Conception		
Spontaneous	62 (88.6)	30 (100)
OI/IUI/IVF	8 (11.4)	0 (0)
Anemia		
Yes	13 (18.6)	4 (13.3)
No	57 (81.4)	26 (86.7)
Diabetes		
Yes	33 (47.1)	1 (3.3)
No	37 (52.9)	29 (96.7)
Hypothyroid		
Yes	23 (32.9)	7 (23.3)
No	47 (67.1)	23 (76.7)
Previous history of PIH/abruption/IUGR		
Yes	7 (10)	0 (0)
No	63 (90)	30 (100)
Treatment history		
Heparin/aspirin	21 (30)	0 (0)
Nil	49 (70)	30 (100)
Menstrual history		
Irregular	10 (14.3)	0 (0)
Consanguinity		
Yes	7 (10)	3 (10)
Mother history of HTN		
Yes	29 (41.4)	8 (26.7)
Father history of HTN		
Yes	15 (21.4)	6 (20)
Nuchal translucency scan		
Normal	65 (92.9)	30 (100)
Not done	5 (7.1)	0 (0)
FTS		
Intermediate risk	2 (2.9)	0 (0)
Low risk	59 (84.3)	22 (73.3)
Not done	9 (12.9)	8 (26.7)
Anomaly scan		
IUGR/Skeletal dysplasia	4 (5.7)	1 (3.3)
Normal	66 (94.3)	29 (96.7)
Mode of delivery		
LSCS	44 (62.9)	12 (40)
Vaginal	26 (37.1)	18 (60)
Previous CS	14 (31.8)	3 (25)
Indication for LSCS		
PE/IUGR/Doppler changes/abruption	14 (31.8)	Nil
Other obstetric indications	16 (36.4)	9 (75)
Vaginal delivery		
Spontaneous	17 (65.4)	5 (27.8)
Induced	9 (34.6)	13 (72.2)
Mean uterine artery Doppler		
High resistance	24 (34.3)	3 (10)
Normal	46 (65.7)	27 (90)
PIH/pre-eclampsia		
Yes	35 (50)	3 (10)
No	35 (50)	27 (90)

IUGR: Intrauterine growth restriction, PIH: Pregnancy-induced hypertension, LSCS: Lower segment cesarean section, PE: Pre-eclampsia, HTN: Hypertension

Table 1: Represents the highest and lowest risk factors for all the parameters

Parameters	Study group		Control group	
	Mean	**SD**	**Mean**	**SD**
Age	28.47	4.66	25.87	3.04
Marital history of	4.73	3.87	3.10	3.23
GA@Delivery	35.44	4.16	38.12	1.21
Birth weight	2.44	0.80	2.96	0.33
APGAR1	7.39	0.97	7.73	0.64
APGAR5	8.59	0.73	8.90	0.31
sFLT-1	826.17	251.31	924.69	360.61
PlGF	23.07	4.68	27.43	5.62
sFLT-1/PlGF ratio	39.68	22.77	35.00	16.98

SD: Standard deviation, sFLT-1: Soluble fms-like tyrosine kinase-1, PlGF: Placental growth factor

From Table 1 in the high-risk patients, the mean age was 28.47 years with a standard deviation (SD) of 4.66 years, the mean gestational age of delivery was 35 weeks+4 days (SD±4.1), and the mean birth weight was 2.44 kg (SD±0.80). In low-risk group, the mean age was 25.87 years with a SD of 3.04 years, the mean GA of delivery was 38 weeks+1 day (SD±1.2), and the mean birth weight was 2.96 kg (SD±0.33). The APGAR scores in both groups are 7/10 and 8/10 at 1 min and 5 min, respectively.

In our study, the mean sFLT-1 in high-risk group was less that is 826.17 ng/L (SD±251.31) as compared to 924.69 ng/L (SD±360.61) in low-risk groups. The mean PlGF in high-risk group was less than 23.07 ng/L (SD±4.68) compared to 27.43 ng/L (SD±5.62) in low-risk group. The mean sFLT-1/PlGF ratio was increased in high-risk group of about 39.68 (SD±22.77) compared to 35 (SD±16.98) in low-risk group.

Among 70 labeled high-risk patients, four patients were expelled before the period of viability; hence, they have been removed from the final analysis in outcome of the pregnancy. In 96 study populations, 30 patients in high-risk group and three in low-risk group have developed GHTN and/or PE.

Development of pre-eclampsia/GHTN					
Risk	**GHTN/ Pre-eclampsia (%)**		**Total (%)**	**P-value**	**Odds ratio (95% confidence interval)**
	Yes	**No**			
High	30 (45.45)	36 (54.54)	66 (100)	0.001	7.5 (2.07–27.18)
Low	3 (10)	27 (90)	30 (100)		
Total	33 (34.37)	63 (65.62)	96 (100)		

GHTN: Gestational hypertension

Considering the risk of the subjects with PE distribution, 45.45% of the high-risk patients had developed PE when compared to low-risk patients of whom 10% had developed PE and the difference was statistically significant (P<0.05). Subjects with high risk have 7.5 odds of getting PE compared to those with low risk.

Values of sFLT-1, PlGF, and sFLT-1/PlGF ratio in the study group

Characteristic	Pre-eclampsia		P-value by "t" test
	Yes	No	
sFLT-1	989.3 (±346.94)	785.97 (±235.3)	0.001
PlGF	21.94 (±5.64)	25.61 (±4.92)	0.001
sFLT-1/PlGF ratio	49.72 (±27.39)	32.51 (±14.89)	0.002

sFLT-1: Soluble fms-like tyrosine kinase-1, PlGF: Placental growth factor

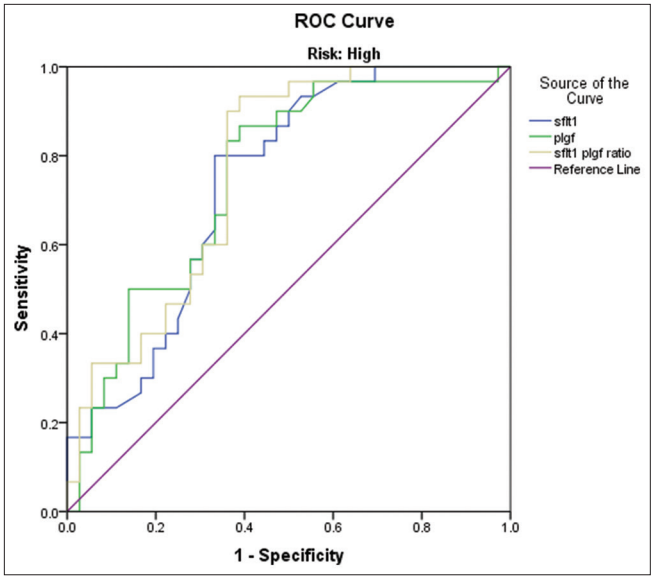
If we consider only PE without gestational HTN, the mean sFLT-1 among those with PE was 989.3 (±346.94) which is higher than the mean sFLT-1 among those without PE which was 785.97 (±235.3) and the difference was statistically significant. The mean PlGF among those with PE was 21.94 (±5.64) which is lower than the mean PlGF among those without PE which was 25.61 (±4.92) and the difference was statistically significant. The mean sFLT-l/PlGF ratio among those with PE was 49.72 (±27.39) which is higher than the mean sFLT-l/PlGF ratio among those without PE which was 32.51 (±14.89) and the difference was statistically significant. Since the P-value is 0.05 in this study, it shows significant values of 0.001 for sFLT-l, 0.001 for PlGF, and 0.002 for sFLT-l/PlGF ratio.

Uterine Doppler in high-risk

Uterine Doppler	Pre-eclampsia (%)		Total (%)	P-value	Odds ratio (95% confidence interval)
	Yes	No			
High resistance	17 (70.83)	7 (29.16)	24 (100)	0.001	8.5 (3–24.07)
Normal	16 (22.22)	56 (77.77)	72 (100)		
Total	33 (34.37)	63 (65.62)	96 (100)		

Considering the uterine Doppler of the subjects with PE distribution, 70.83% of the high resistance uterine Doppler had PE which is higher compared to normal uterine Doppler of whom 22.22% had PE and the difference was statistically significant (P<0.05). Subjects with high resistance uterine Doppler have 8.5 odds of getting PE compared to those with normal uterine Doppler.

Receiver operating characteristics curves for predicting pre-eclampsia



Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these tests in high-risk group

If we keep the cut off of sFLT-1 for PE as 771.87 ng/L, the sensitivity becomes 80% (68.6–87.9) and the specificity is 67.6% (55.4–77.7). PPV is 57.14% and NPV is 63.16%. Similarly, for PlGF, if we keep the cut off as 25.96 ng/L, the sensitivity is 80% and PPV is 60% and specificity is 63.9% and NPV is 66.67%. Moreover, if the sFLT-1/PlGF ratio is 32.25, the sensitivity is 90% (82.5–94.4) and the specificity is 63.9% (54.1–72.6). PPV is 62.86% and NPV is 74.19%.

Secondary outcome

Among the high-risk patients, 14 patients showed intrauterine growth restriction (IUGR) with or without Doppler changes. Out of these 14 patients, nine of them had developed GHTN/PE and six of them had uterine artery high resistance and their biochemical markers are as follows: Mean sFLT-1 value was 857.37 ng/L, PlGF was 23.32 ng/L, and sFLT-/PlGF ratio was 37.50.

DISCUSSION

This study evaluated the role of the sFLT-1/PlGF ratio along with uterine artery Doppler for the prediction of PE and also for IUGR as a secondary outcome. HDP has a major role in maternal health worldwide and contributes to 9–25% of deaths worldwide.[11] It is the second leading cause for maternal mortality ratio in India with the incidence of HDP of about 14% in that PE contributes 2–5%.[12] Hence,

Biomarkers	Area under curve (95% confidence interval)	P-value	Cutoff	Sensitivity (%)	Specificity (%)
sFLT-1	0.69 (0.58–0.79)	0.002	771.87	80 (68.6–87.9)	67.6 (55.4–77.7)
PlGF	0.75 (0.64–0.85)	0.001	25.96	80 (68.6–87.9)	63.9 (54.1–72.6)
sFLT-1/PlGF	0.76 (0.66–0.85)	0.001	32.25	90 (82.5–94.4)	63.9 (54.1–72.6)

we would like to identify a better screening tool to predict the development of PE in high-risk mothers so that we can anticipate complications and manage them accordingly. As there are many consensus that is showing an imbalance between angiogenic and anti-angiogenic factors that are responsible for the development of PE,[2-11] we focused on them to identify the better marker for its screening.

As we are trying to predict the occurrence of PE with the help of these biomarkers, the sensitivity of the study is 90% (82.5–94.4) and the specificity is 63.9% (54.1–72.6) if we keep sFLT-1/PlGF ratio as ≥32.25 which is similar to case-controlled study by Taraseviciene et al.,[2] 2016, that showed significantly higher levels of sFlt-1, sFlt-1/PlGF ratio, and mean UAPI and UARI and lower levels of PlGF in PE group when compared to controls. The highest sensitivity and specificity for PE had SFlt-1/PlGF ratio and PlGF with the cut-off values of ≥35 (sensitivity of 95.8% and specificity of 96.2%, respectively) and ≤138.6 pg/mL (sensitivity of 95.8% and specificity of 93.7%, respectively). We have also observed the intrauterine fetal growth restriction as a secondary outcome and calculated these values in that specific group and found that the mean sFLT-1 value was 857.37ng/L, PlGF was 23.32ng/L, and sFLT-/PlGF ratio was 37.50 which is similar to Chang et al.,[3] study. Due to limited resources, we are able to collect only one sample for measuring these values unlike Khalil et al.,[4] study. They have found that sFLT-1 levels are increased from 15-week gestation onward and PlGF levels are decreased from 11-week onward. They have concluded that in screening for preterm PE, maternal serum level of PlGF is a useful marker from the first trimester onward while sFLT-1 level is likely to have a predictive value from the second trimester onward.

Hassan et al.,[8] 2013, have measured levels of sFlt-1, PlGF, and sFlt-1/PlGF ratio at mid-trimester in 83 women who developed PE matched with 250 controls. In their study, they found that the sFlt-1/PIGF ratio at a cut-off value of 24.5 was more effective for prediction of PE with the highest sensitivity, specificity, and accuracy of 91.6%, 86.4%, and 87.7%, respectively, with OR 67. Parra–Cordero et al.,[9] 2013, have done a nested case–control study involving 5367 asymptomatic pregnant women undergoing routine transvaginal uterine artery Doppler at 11 weeks to 13+6 weeks. Following exclusions, 70 pregnant women who later developed PE and 289 control patients enrolled during the first trimester who had serum or plasma samples taken at enrolment. They have found that an increased lowest UtA-PI was significantly associated with both early- and late-onset disease. PlGF MoM was significantly reduced in women who later developed PE. Logistic regression models which include maternal characteristics, PlGF and UtA-PI, can predict approximately half of the pregnancies that will be complicated by early-onset PE. Basuni et al.[10] in 2012, have done a study on 88 pregnant women by doing SFLT-1, PlGF along with other blood investigations. They found that there was a highly statistically significant difference between mild and severe cases, where the sFLT-1 increased with increased severity of PE (P<0.001). Receiver operating characteristics study showed that the best cut-off value for sFLT-1 was 2075 pg/mL and the sensitivity was 75%, whereas the specificity was 85%. The cut-off value for PlGF was 151 ng/mL; the sensitivity was 75%, whereas the specificity was 72.1%. There was a highly statistically significant negative correlation between PLGF and sVEGFR-1.

Strengths of the study

1. Prospective longitudinal study including maternal characteristics and history
2. We are able to get the maternal and neonatal outcome
3. We are able to set a cut off for these biomarkers with a good sensitivity rate and NPV.

Limitations of the study

1. Relatively small sample size
2. It is difficult to apply as a standard screening tool for all pregnant women unless they have strong risk factors for developing PE.

CONCLUSION

The study concluded that the significant higher levels of sFLT-1, SFLT-1/PlGF ratio increased mean uterine artery PI and RI, and lower levels of PlGF are associated with PE. These biomarkers should be used as a diagnostic tool for predicting PE along with other high-risk factors if present in the history. Moreover, it also showed that significant altered values were associated with adverse pregnancy and neonatal outcomes.

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Authors Contribution:
SJ- Introduction, literature survey, prepared a first draft of the manuscript, implementation of the study protocol, data collection, data analysis, manuscript preparation, and submission of an article; **KT-** Of study, statistical analysis, interpretation, and review manuscript; **SP-** Review manuscript, literature survey, and preparation of figures; **JV-** Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **STP-** Coordination and manuscript revision

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