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Predictive Ability of Ratio of Soluble Fms Like Tyrosine Kinase-1 And Placental Induced Growth Factor (sflt-1/PlGF) In Diagnosis of Preeclampsia: A Case Control Study.

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ABSTRACT

Preeclampsia (PE) is a complicated disorder which is characterized by high blood pressure and proteinuria or the presence of end-organ dysfunctioning mostly occurring after 20 weeks of pregnancy. It is estimated that this condition affects between 2 to 5% of pregnancies globally. It is a systemic syndrome that originates from placenta and is associated with an imbalance between angiogenic factors in the maternal circulation. Now a days, anti-angiogenic factor, namely sFlt-1 (Soluble fms-like tyrosine kinase 1) and PlGF (Placental induced growth factor) which is a pro-angiogenic factor, have been proposed as promising biomarkers for predicting the occurrence of preeclampsia (PE). Soluble fms-like tyrosine kinase 1 (sFlt-1) and PlGF are remarkably disturbed in preeclampsia. The ratio of maternal serum sFlt-1 to PlGF(sFlt-1/PlGF) has been used to rule out the occurrence of disease. The aim of this study is to highlight the predictive role of sFlt-1/PlGF ratio with regards to preeclampsia development in third trimester of pregnancy.

Methods: A comparative study was made on 40 women with PE and 40 age-gestational-age matched women without PE, as case and control, respectively. Levels of sFlt-1, PlGF and the ratio of sflt-1/PlGF were compared between the two. Receiver Operating Characteristic (ROC) curve analysis was done to calculate diagnostic accuracy of sFlt-1/PlGF ratio.

Results- – Serum level of sFlt-1 was significantly higher in preeclamptic patients (3487.58±1543.46pg/ml) than in controls (2824.67±1050.24pg/ml) whereas PlGF was significantly lower in preeclamptic patients (78.54±152.63pg/ml) than controls (553.73±263.27pg/ml). The mean sflt-1/PlGF ratio in cases was 78.54±40.37pg/ml which is higher than mean sFlt-1/PlGF of controls which was 37.14±149.70. When ROC analysis was done sFlt-1/PlGF>22.53 was found giving highest Youden index with sensitivity of 100% and specificity of 94.87 and AUC=0.94.

Conclusion-This study looked at the evaluation sFlt-1 levels, serum PlGF levels and their ratio sFlt-1/PlGF and judging diagnostic accuracy of sFlt-1/PlGF in preeclamptic females in the third trimester of pregnancy. The level of sFlt-1 was higher in preeclamptic female whereas PlGF level was low and ratio sFlt-1/PlGF was also on the higher side. Hence the combined measurement and calculation of their ratio may help in making better diagnosis and more specific disease management.

Keywords: Preeclampsia, ratio, normotensive.

INTRODUCTION

Preeclampsia is a multisystemic condition peculiar to pregnancy and is characterized by endothelial dysfunction [1]. This disease manifests in 5% to 8% of all pregnant females and remain a problem. It is an important contributor to maternal and fetal morbidity and mortality throughout the world [2]. The possible cause of preeclampsia is defective remodelling of spinal arteries which causes hypoperfusion and systemic dysfunction [3]. This further causes imbalance of circulating proangiogenic and antiangiogenic factors [4,5].

The etiology of PE has not been fully elucidated. There is evidence that maternal endothelial dysfunction due to placental factors plays a significant role in the pathogenesis of PE [6,7]. Soluble fms-like tyrosine kinase-1 (sFlt-1), an anti-angiogenic factor secreted by the placenta, binds to vascular endothelial growth factor (VEGF) and placental induced growth factor (PlGF) in the maternal circulation. In the bound form, sFlt-1 interacts with membranous tyrosine kinase, which is critical to the biological activity of sFlt-1. High concentrations of antiangiogenic factors (e.g., sFlt-1) and low concentrations of proangiogenic factors (e.g.,VEGF and PlGF) can produce an antiangiogenic state, leading to general maternal vascular dysfunction[8, 9] and eventually to hypertension, proteinuria, and other clinical manifestations of PE[10].

At present, the biggest problem in clinical practice is failure to identify patients with preeclampsia early. Patients are already in the middle or late stages of the disease when treated, often have multiple concurrent organ complications, and need referral to a tertiary care centre or multidisciplinary treatment [11]. However, if we can detect and identify high-risk PE patients early, we may be able to reduce the incidence of PE and prevent the occurrence of maternal and fetal complications [12]. Due to the complex pathophysiological
characteristics and clinical unpredictability of PE and limited evidence for the detective performance of different diagnostic methods, there has been no accurate and reliable diagnostic method for predicting PE till date [13, 14].

Recently, angiogenic factors like sFlt-1 & PlGF are considered as good candidate biomarkers for prediction of preeclampsia [15].

It has been observed that in preeclampsia serum sFlt-1 get increased and PlGF get decreased and the resultant sFlt-1/ PlGF ratio tend to increase [16,17]. The disturbances in these angiogenic factors occurs prior to onset of other clinical symptoms hence helpful in screening out females with high risk for preeclampsia from those having normal pregnancies.

The entire focus of research is based on to investigate the role of these biomarkers in pathogenesis and development of preeclampsia. Hence estimation of these two markers i.e. sFlt-1 and PlGF and especially their ratio (sFlt-1/PlGF) may play a better role than single marker in prediction of preeclampsia [17,18,19].

Material and method

This comparative study was carried out in the Department of Biochemistry of SMS Medical College & Hospital, Jaipur (Rajasthan) from April 2023 to August 2023. During the study period, pregnant

women with viable singleton fetus in their third trimester, diagonosed with preeclampsia were taken as case group and non-preeclamptic pregnant female who were matched for gestational and biological age were taken as controls. Patients were recruited from antenatal clinics, wards, and emergency units by purposive sampling. The study was approved by the Institutes Ethics Committee (Reference No147MC/EC/2023 Dated 01/04/2023), and informed written consent was obtained from all the participants.

Inclusion Criteria:

1. Pregnant females with gestation >28 weeks.
2. All pregnant subjects with singleton pregnancy who were willing to participate.
3. All pregnant women who gave informed consent.

Exclusion Criteria:

1. Pregnant females with previous history of hypertension.
2. Severely ill patients.
3. Pre-existing diagnosed preeclampsia or haemolysis.
4. Pregnant women with renal diseases.
5. Pregnant females with multiple fetuses.
6. Pregnant women with gestational diabetes.
7. Pregnant women with chronic hypertension.
8. Pregnant women who didn't give informed consent.

SAMPLE SIZE:

Sample size was calculated at 80% study power and alpha error of 0.05 assuming SD of 92.43 for sFlt-1/PlGF ratio in preeclamptic patients as found in the study of Abidoye Gbadegesin et al., (DOI: 10.4236/ojog.2021.116070 June 18,2021 Open Journal of Obstetrics and Gynaecology).

For minimum detectable mean difference of 60 in sFlt-1/PlGF ratio between preeclamptic patients and normotensive patients, 37 patients in each group were required a sample size for previous study which is further enhance and rounded off to 40 patients in each group as final sample size expecting 10% attrition.

Formula used for the calculation of sample size,

$$n = \frac{2(Z_{1-\alpha/2}+Z_{1-\beta})^2\sigma^2}{(M_1-M_2)^2}$$

n = sample size

$Z_{1-\alpha/2}$ = 1.96 (corresponding 'Z' value for α error of 0.05)

$Z_{1-\beta}$ = 0.84 (corresponding 'Z' value for % study power)

σ =assumed standard deviation^2

Matching: Preeclamptic(cases) and non-preeclamptic group(controls) were matched for their gestational and biological age to eliminate confounding effect.

The blood pressure of control group was monitored to make sure they remained normotensive during pregnancy otherwise were excluded.

Methodology-All eligible patients who visited Mahila Chikitsalya, SMS Medical College, Jaipur, during study period were screened through inclusion and exclusion criteria was approached by the

investigator herself and explained about nature and purpose of study through participant information sheet (PIS).

After taking detailed history of participants general and obstetrics examination was done by co-guide of study and required investigations was done.

Blood samples were taken at recruitment from each participant. 5ml of venous blood has collected from the patients into plain bottles that were well labelled for prior identification, left for two hours to clot, and retract, and then centrifuged at 2500RPM for 5 minutes. The serum was decanted into vials and stored at -80 ◦C until analysis. Maternal serum PIGF and sflt-1 assayed using ELISA (sandwich principle) according to manufacturer's instructions. For serum PIGF and sFlt-1 assay, kit provided by Elabsciences and Sunlong Research and Diagnostics products were used respectively. All tests assayed on Bio-Tek ELISA machine provided by Department of Biochemistry, SMS Medical College and Hospital, Jaipur.

Patients with blood pressure >140/90 mmHg with proteinuria ≥2 considered as preeclamptic patients, and they constitute group A as case group.

Gestational and biological age matched pregnant women were taken as control group and constituted group B.

Data Analysis-

All information/data/ finding thus collected was recorded on a pre-design study Performa and entered in MS-Excel sheet to prepare master chart. This master chart was subjected to statical analysis. Qualitative variables were summarised as mean and SD whereas quantitative variables were presented as proportional (%).

Unpaired T-test was used for analysis of quantitative variable while Chi-square test was applied for analysis of qualitative variables.

P-value <0.05 was taken as significant.

Medcale 16.4 version software has been used for all statistical calculations.

RESULTS

Participants Characteristics-

In present study, mean age of cases was (27.05±34.35years) while that in control group was (26.80±32.22years).47.50 % of cases and 32.50 % of controls were second gravida while 20 %&25% of participants were primigravida among cases and controls respectively. Both the groups were found comparable with respect to age, gravida, and parity.

Levels of Angiogenic Factors in Preeclamptic Women-

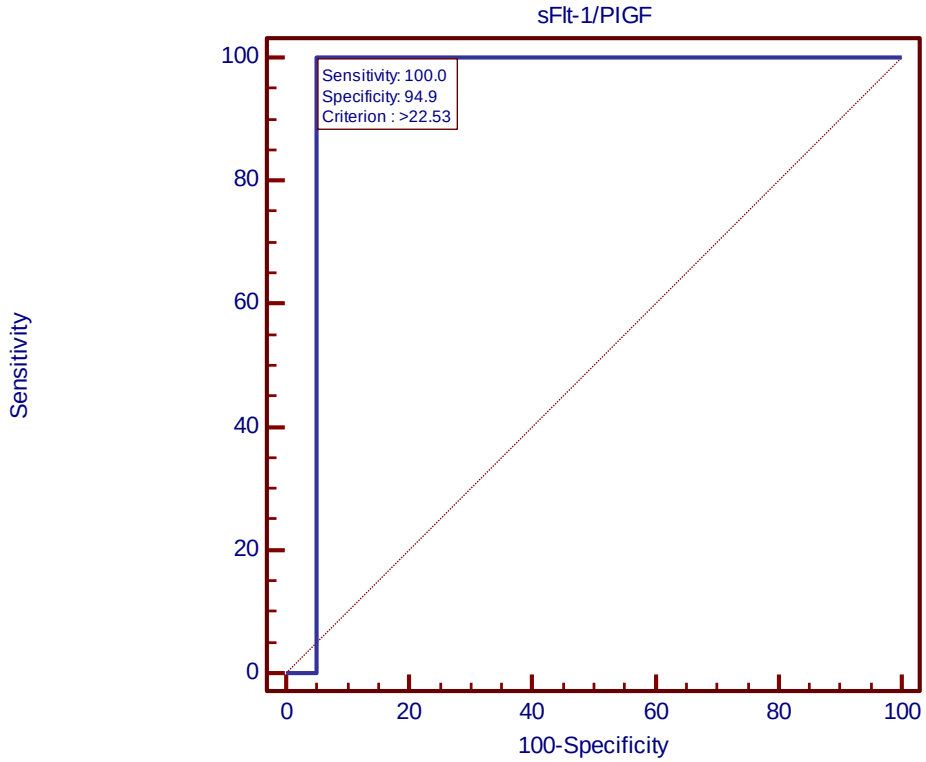
Parameters	Group	N	Mean	SD	Median	Min.	Max.	'p' Value*
PIGF	A	40	78.54	152.63	53.7	0	1000	<0.001
	B	40	553.73	263.27	583	139	1000	
sFlt-1/PIGF	A	37	78.35	40.37	66.5	23.83	201.89	0.110
	B	39	37.14	149.70	5.92	0.58	900	
sFlt-1	A	40	3487.59	1543.46	3253.75	900	9160	0.028
	B	40	2824.67	1050.24	2918.25	480.75	4627	

Table No :1

Mean PIGF levels in cases were 78.54±152.63pg/ml which were significantly lower than controls 553.73±263.27 pg/ml.

Mean sFlt-1 levels in cases were (3487.59±1543.46pg/ml) which were significantly higher than controls (2824.67±1050.24pg/ml).

Similarly, sFlt-1/PlGF ratio in cases was 78.54±40.37 which was non-significantly higher than controls 37.14±149.70 pg/ml.



Area under the ROC curve (AUC)	0.949
Standard Error	0.0358
95% Confidence interval	0.873 to 0.986
z statistic	12.540
Significance level P (Area=0.5)	<0.0001

Table No :2

ROC Curve Analysis

When ROC curve analysis was done to find out optimum cut-off value of serum sFlt-1/PlGF ratio to discriminate cases from controls, it was found that sFlt-1/PlGF ratio >22.53pg/ml was showing 100.0% sensitivity (95% CI-80.0-100) and 94.87% specificity (95%CI-82.7-99.4) with AUC of 0.94(95% CI =0.87-0.98). (Table.2).

DISCUSSION

This study was designed to evaluate the blood levels of PlGF and sFlt-1 and their ratio sFlt-1/PlGF, in pregnant females diagnosed with preeclampsia (cases) and compared to pregnant females without preeclampsia (controls) in their third trimester of pregnancy. Additionally, the study explored the possibility of sFlt-1/PlGF as a predictive marker for preeclampsia.

sFlt-1 and PlGF in preeclampsia development-

In our study, the mean levels of sFlt-1 in cases were significantly higher as compared to controls.
In a similar study done by Hertig et al.,[20] mean serum sFlt1 concentrations were significantly higher in women with preeclampsia than in those with normotensive pregnancies. Similarly, in a study done by Abidoye et al.,[21] where also maternal serum sFlt-1 was higher in PE compared with normotensive pregnancies but the stastical difference was non-significant.
In our study mean PlGF levels were significantly low in cases in comparison to controls. This was in concordance with the study done by Abidoye et al.,[21] where also the mean serum PlGF were significantly lower in the PE group compared to normotensive pregnancies.
Similarly, Levine et al.,[16] also find same conclusion in their study where the PlGF levels were significantly lower in the preeclamptic pregnant women than in the normotensive controls.

sFlt-1/PlGF Ratio in preeclampsia development-

In our study, the ratio of sFlt-1/PlGF was non-significantly high which is in accordance with the study done by Anne Karge et al.,[22] where also sFlt-1/PlGF ratio was non-significantly high. Similarly, in a study done by Abidoye et al.,[21]and Shuyuan Xue et al.,[23] the ratio sFlt-1/PlGF ratio was high, but the statistical difference was significant.
Present study finds a good predictive role of serum sFlt-1/PlGF ratio (AUC=0.94) in early diagnosis of PE similar to the study of Pooneh et al.,[19] and Lim et al.,[24] where they also found positive predictive role of sFlt-1/PlGF (AUC=0.90 & AUC=0.85 respectively) for PE. However, sensitivity and specificity for corresponding cut-off value were not coherent with the present study.

Limitations-First limitation of this study was the sample size. Many More studies may be required to determine the effectiveness of sFlt-1/PlGF ratio as a biomarker for PE diagnosis in larger population. One more limitation was that it was not possible to carry out sampling before the diagnosis of PE and samples were taken once the diagnosis was made.

Conclusion- Our results demonstrate that the use of sFlt-1/PlGF ratio may help in making better predictive role for clinical diagnosis of preeclampsia and may enable better patient management. Using this ratio might lead to more precise screening and faster diagnosis of preeclamptic females thus preventing serious maternal and fetal complications.

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