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Angiogenic Biomarkers (sFlt / PLGF): An Approach for Clinical Integration of Pre-eclampsia

Gehad Mohsen Mohamed El-Mazny[1], Ekbal Mohamed Abo-Hashem[1], Mona Abo Bakr El-Hussiny[1], Shereen Mamdouh Abdelwakil Mahammed[2], Mostafa Mohamed Mansour[1]

[1]Clinical Chemistry Unit, Clinical Pathology Department and

[2] Department of Obstetrics and Gynecology, Mansoura, Faculty of Medicine, Mansoura University, Egypt.

Corresponding author: Gehad M. El-Mazny, Phone number: +201007222319, Email: gehadmohsen422@gmail.com

ABSTRACT

Background: One of the most important pregnancy problems is pre-eclampsia (PE). PE is caused by changes in the circulating levels of angiogenic agents. Endothelial dysfunction is caused by elevated levels of antiangiogenic factors like soluble fms-like tyrosine kinase-1(sFlt-1) and decreased levels of angiogenic factors like placental growth factor (PlGF). **Objective:** This study's objective is to assess the significance of alternation of angiogenic balance as a pre-eclamptic marker and their association to PE severity.

Patients and Methods*:* One hundred pregnant women in third trimester participated in this case-control study, with 40 having mild PE, 40 having severe PE, and 20 healthy pregnant ladies as control. It was done in Clinical Pathology and Obstetrics and Gynecology Departments of Mansoura University Hospitals during a period from "November 2020 to April 2022". Clinical and laboratory tests on all individuals were performed, including CBC, LDH, sFlt-1 in the serum, PLGF, liver function tests, renal function tests.

Results: In comparison to the control group, greater levels of sFlt-1 and sFlt-1/PLGF and lower levels of PLGF were present in all cases of mild and severe PE (p0.001 for each). Additionally, severe cases demonstrated significant high levels of sFlt-1 and sFlt-1/PLGF and significant low levels of PLGF with superior AUCs when compared to moderate instances (AUC=0.999).

Conclusion: sFlt-1, PLGF and sFlt-1/PLGF ratio may have a role in the prediction of severity of PE, and thus diagnosis of PE, thus may be used in management of PE.

Keywords: Angiogenic biomarkers, Pre-eclampsia, PLGF, sFlt-1.

INTRODUCTION

Around 5-8% of pregnancies worldwide experience PE, a risky pregnancy condition characterised by hypertension and harm to numerous maternal organs. Particularly the early onset variety is a major contributor to maternal and neonatal morbidity and mortality**(1)**.

The onset and development of the diseases brought on by PE are significantly influenced by the placenta. PE is caused by a combination of genetic, immunological, and angiogenic factors. PE appears to have its roots in the defective trophoblast cell invasion of the mother's spiral arteries during localised aberrant immunological contacts between the fetoplacental unit and mother. Chronic hypoxia is brought on by placental underperformance. The placenta experiences apoptosis and necrosis as a result of increased local oxidative stress brought on by the reoxygenation that takes place in conjunction with it. Apoptotic bodies released into the mother's circulation, pro-inflammatory and antiangiogenic placental mediators, and dysfunctional maternal systemic endothelial cells are all related to systemic inflammation**(2).**

Preeclampsia is caused by pathogenic changes in the circulating angiogenic factors. Angiogenesis, the process by which existing blood arteries are used to create new ones, is carefully regulated by angiogenic agents. Additionally crucial for maintaining healthy vessels, angiogenic factors serve as critical cues for organ development. VEGF, PlGF, the antiangiogenic factors sFlt-1 and soluble Endoglin (sEng), capture transforming growth factor beta (TGFb) in the bloodstream, respectively. This lowers their free levels, results in endothelial dysfunction, and exacerbates the disease's clinical symptoms **(3).**

As a result, this study aims to assess the importance of angiogenic factor imbalance (sFlt-1/PLGF) "as a marker for PE" and assess their association to PE severity.

PATIENTS and METHODS

The current investigation, a case control study, was conducted in the Clinical Pathology Department and Obstetrics and Gynecology Department, Mansoura University Hospitals during a period from "November 2020 to April 2022".

This study included 100 pregnant women in child bearing period (aged 19-44 years) with a gestational age of 24-36 weeks and six days of amenorrhea. They were classified into 3 groups:

Group I: included forty pregnant patients (in 3rd trimester) with mild PE, which was divided into 14 patients with GA <34 weeks and 26 patients with GA ≥34 weeks.

Group II: forty patients (in 3rd trimester) with severe PE, which was divided into 7 patients with GA <34 weeksand 33 patients with GA ≥34 weeks.

Group III: twenty healthy pregnant ladies acting as control with GA ≥34 weeks.

Twin pregnancies, pregnancies with less than twenty-four weeks of gestation, and pregnancies in

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which the mother had any chronic condition (such as systemic lupus erythematosus (SLE), diabetes mellitus, preexisting hypertension, chronic heart disease, or chronic renal disease) were all excluded from the study.

All cases underwent thorough clinical examinations, with a focus on body weight and blood pressure monitoring, a standard obstetric assessment, and radiological examinations including: foetus evaluation and abdominal ultrasound.

CBC, liver function tests, creatinine, LDH, uric acid, and urine protein (24 hour collected sample) tests were carried out. Serum sFlt-1, PLGF, and (sFlt-1/ PlGF) ratio were assessed by using an ELISA kit provided by (Glory Science Co. Ltd)/China as a special laboratory test in the prediction and diagnosis of placental dysfunction.

Serum FTL1 and serum PLGF:

- **Principle of the test (Sandwich ELISA Protocol):**

The kit for determining the amount of PLGF/FTL1 in a sample quantitatively uses purified human PLGF/FTL1 to make solid phase antibodies, add purified human PLGF/FTL1 to wells.Purified human PLGF/FTL1 antibody should be combined with labelled HRP to create an antibody antigen - enzyme antibody complex, which should then be thoroughly washed before TMB substrate solution is added to colour the substrate blue at 450 nm. The application of PLGF/FTL1 in the trial was then calculated by associating the Optical Density (O.D) of the trials to the average curve.

Ethical Approval:

The ethics approval and written agreement to participate in the study have been signed by all patients and controls. The Institutional Review Board (IRB), Mansoura University MD.19.01.127, Faculty of Medicine, authorized the study. All authors have read the author rules and gave their agreement for this work to be published. The Declaration of Helsinki and the code of ethics of the World Medical Association, was followed when conducting this research on humans.

Statistical analysis:

The Statistical Package for the Social Sciences (Armonk, New York: IBM Corp.), version 25.0 of IBM SPSS Statistics for Windows was used for statistical analysis. The acquired data were edited, coded, and tabulated. It was determined whether the data distribution was normal by using the Kolmogorov-Smirnov test. The Mann Whitney test (U test) was used to measure the arithmeticalimport of the difference between two research groups for a non-parametric variable. The receiver operating characteristic (ROC) curve was used tool for evaluating the sensitivity and specificity of quantitative diagnostic tests that separate cases into two groups. Logistic regression analysis was applied to determine risk factors. P value $\leq$ 0.05 with a 95% confidence interval was considered significant.

RESULTS

Total, mild and severe PE cases showed significantly higher sFlt-1, sFlt-1/PLGF (Fig. 1and 3 respectively), significantly lower PLGF (Fig.2) when compared to control group. In addition, severe cases showed significantly higher sFlt-1, sFlt-1/PLGF, and significantly lower PLGF when compared to mild cases.

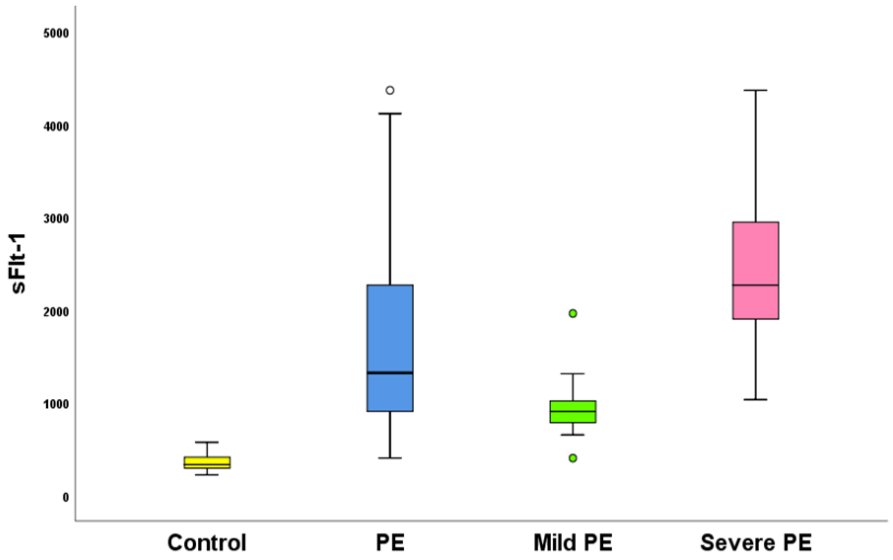

Figure (1) sFlt-1 among studied subjects.

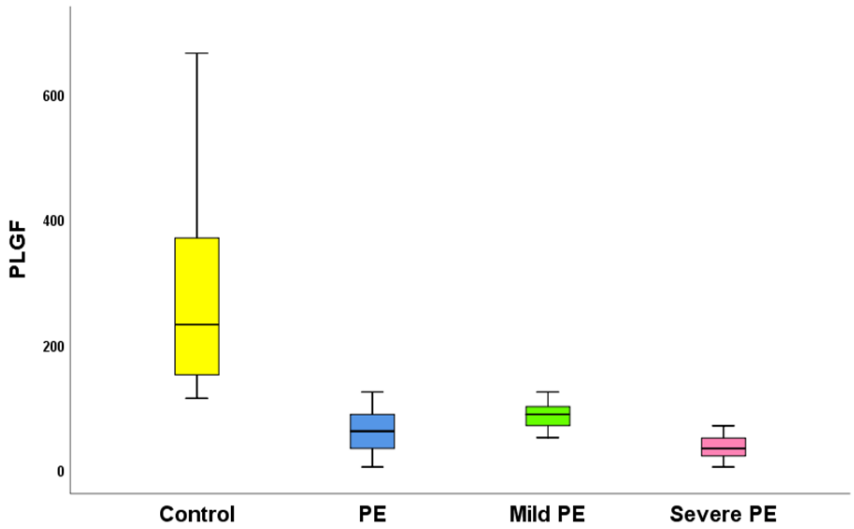

Figure (2) PLGF among studied subjects

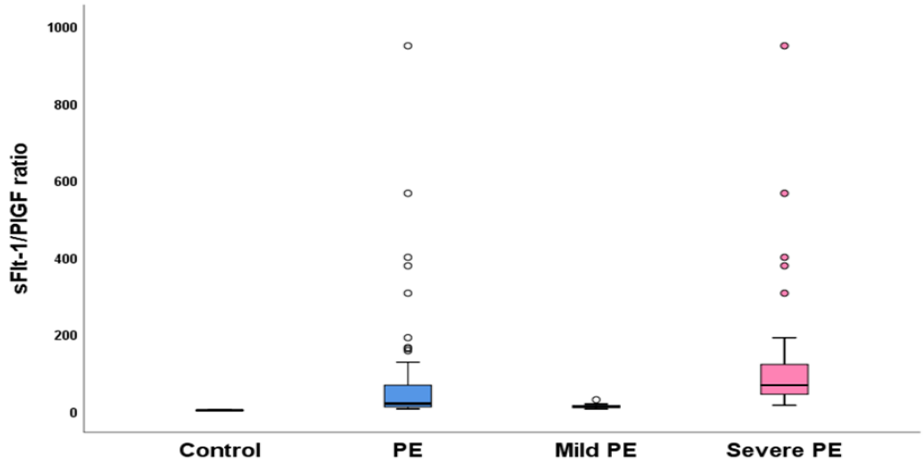

Figure (3) sFlt-1/PLGF among studied subjects.

Excellent AUCs were displayed by sFlt-1 and PLGF. While sFlt-1/PLGF ratio showed perfect AUC for discrimination between PE and healthy subjects. PLGF, sFlt-1 had excellent AUCs. Moreover, their combination improved AUC for diagnosis of PE (i.e., increased their power of ability for PE diagnosis) (Table 1).

Table (1) Performance characteristics of PLGF, sFlt-1, sFlt-1/PLGF ratio for discrimination between PE cases and healthy control group

Parameter	sFlt-1	PLGF	sFlt-1/PLGF
AUC	0.997	0.997	1
95% CI	0.990-1	0.99-1	1-1
Cut off	610.4	112.5	4.6
Sensitivity (%)	98.8	96.3	100
Specificity (%)	100	100	100
PPV (%)	98.8	96.3	100
NPV (%)	100	100	100
Accuracy (%)	99	97	100

AUC: area under the receiver operating characteristic curve; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval.

ROC curves of sFlt-1, PLGF and sFlt-1/PLGF ratio were conducted for discrimination between mild and severe PE cases. All markers showed excellent AUCs. Best cut off values and performance characteristics are shown in table (3). PLGF, sFlt-1 had excellent AUCs. Moreover, their combination improved AUC for prediction of severity (i.e., increased their ability for prediction of PE severity) (Table 2).

Table (2) Performance characteristics of PLGF, sFlt-1, sFlt-1/PLGF ratio for discrimination between mild and severe PE cases

Parameter	sFlt-1	PLGF	sFlt-1/PLGF
AUC	0.987	0.976	0.993
95% CI	0.967-1	0.951-1	0.981-1
Cut off	1319.1	66.5	19
Sensitivity (%)	97.5	95	97.5
Specificity (%)	97.5	87.5	97.5
PPV (%)	97.4	95.0	97.5
NPV (%)	97.5	87.5	97.5
Accuracy (%)	97.5	91.3	97.5

AUC: area under the receiver operating characteristic curve; PPV:positive predictive value; NPV: negative predictive value; CI: confidence interval

Lower PLGF and higher sFlt-1/PLGF ratio were significantly associated with late onset PE. Higher

miRNA210 and sFlt-1 were non significantly associated with late onset PE (Table 3).

Table (3): Comparison of sFlt-1, PLGF and sFlt-1/PLGF ratio according to onset of PE

Parameter		Early onset PE	Late onset PE	P
		N=21	N=59	
sFlt-1 (pg/ml)	**Median (range)**	966.3 (400.2-2845.3)	1444.7 (650.2-4365.8)	**0.093**
PLGF (pg/ml)	**Median (range)**	67.3 (13.5-120.6)	59 (3.7-123.5)	0.030
sFlt-1/PLGF ratio	**Median (range)**	10.9 (6-164.7)	29.5 (5.4-948.7)	**0.023**

PE: pre-eclampsia.

ROC curves of sFlt-1, PLGF, sFlt-1/PLGF ratio were conducted for discrimination between early onset PE cases and healthy control groups (<34 weeks). sFlt-1 and PLGF showed excellent AUCs, while sFlt-1/PLGF showed perfect AUCs. Best cut off values and performance characteristics are shown in table (4).

Table (4) Comparison of studied parameters between early onset PE cases and healthy control groups (<34 weeks)

Parameter	sFlt-1	PLGF	sFlt-1/PLGF
AUC	0.984	0.992	1
95% CI	0.945-1	0.967-1	1-1
Cut off	644.1	112.5	4.9
Sensitivity (%)	95.2	95.2	100
Specificity (%)	100	100	100
PPV (%)	95.2	95.2	100
NPV (%)	100	100	100
Accuracy (%)	96.3	96.3	100

AUC: area under the receiver operating characteristic curve; PPV:positive predictive value; NPV: negative predictive value; CI: confidence interval.

ROC curves of sFlt-1, PLGF, sFlt-1/PLGF ratio were conducted for discrimination between late onset PE cases and healthy control groups (≥34 weeks). PLGF showed excellent AUCs, while sFlt-1 and sFlt-1/PLGF showed perfect AUCs. Best cut off values and performance characteristics are shown in table (5).

Table (5) Comparison of studied parameters between late onset PE cases and healthy control groups (≥34 weeks)

Parameter	sFlt-1	PLGF	sFlt-1/PLGF
AUC	1	0.999	1
95% CI	1-1	0.995-1	1-1
Cut off	580.2	120	3.96
Sensitivity (%)	100	98.3	100
Specificity (%)	100	100	100
PPV (%)	100	98.3	100
NPV (%)	100	100	100
Accuracy (%)	100	98.6	100

AUC: area under the receiver operating characteristic curve; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

In order to predict PE susceptibility, a logistic regression analysis was done utilizing the angiogenic biomarkersratio as variables. Both were regarded as PE susceptibility predictors in single- and multiple-variable studies (Table 6).

Table (6): Regression analysis for prediction of PE susceptibility

Parameter	Univariable				Multivariable			
	p	**OR**	**95% CI**		***p***	**OR**	**95% CI**	
Angio-genic bio-markers ratio	**<0.001**	1.682	1.068	2.649	**<0.001**	1.723	1.273	1.921

DISCUSSION

PE is a multisystem condition that affects five to eight percent of pregnant women and is still the primary factor in maternal and foetal morbidity and mortality. The aberrant vascular response to placentation that the condition is characterized by means that the biology and causation of PE are yet unknown[4].

PE susceptibility is also greatly influenced by genetic components of angiogenesis and immunological interactions between the mother and fetus. Early-onset or preterm PE, in contrast to late-onset PE, can be predicted more accurately in the first trimester by a combination of maternal characteristics, biophysical indications, and biochemical markers[5]. According to several studies, Angiogenic variables may be useful in distinguishing between diseases such gestational thrombocytopenia and PE and superimposed PE, immune thrombocytopenic purpura, renal illness, chronic hypertension, and pregnant hypertension [6].

In situations of suspected preterm PE, clinical usage of biomarkers like sFlt-1 and PLGF has also recently emerged. Although their sensitivity is moderate, with normal results, their high negative predictive value makes it possible to confidently rule out illness in women. A concerted attempt has been made to find prospective new biomarkers that could enhance prediction[7].

The current study's objective is to assess the significance of angiogenic factor alterations (sFlt-1/PLGF) as a marker for PE and to assess how these changes relate to PE severity, maternal problems, and newborn prognosis. The present study was conducted on 80 cases of PE. Their mean age was 28.9 years. The mean age of mild cases was 28.7 years, while the mean age of severe cases was 29.1 years. In addition to healthy control pregnant females of matched age.

Regarding the validity of PLGF and sFlt-1 in diagnosis and prediction of PE, cases had considerably lower PLGF, compared to the control group, there were significantly lower levels of sFlt-1/PLGF and significantly greater levels of sFlt-1. Additionally, severe PE had significantly lower levels of sFlt-1, the sFlt-1/PLGF ratio, and PLGF than moderate PE did.Additionally, PLGF, sFlt-1, and sFlt-1/PLGF ratio showed excellent AUC for differentiating between groups of patients with mild and severe PE as well as between cases and healthy control groups. Additionally, their combination improved AUC for prediction of severity (i.e., increased their ability for prediction of PE severity). The following values were added to ROC curves of PLGF, sFlt-1, and sFlt-1/PLGF ratio to distinguish between PE patients and control groups(i.e., diagnosis of PE). Excellent AUCs were displayed by PLGF and sFlt-1 (0.997 and 0.997, respectively).While sFlt-1/PLGF ratio showed perfect AUC (AUC=1) for discrimination between PE and healthy subjects. PLGF, sFlt-1 had excellent AUCs. Moreover, their combination improved AUC for diagnosis of PE (i.e., increased their power of ability for PE diagnosis).

Regarding the results of performance characteristics of PLGF, sFlt-1, sFlt-1/PLGF ratio for discrimination between PE cases and healthy control group various authors were in agreement with our results: MacDonald *et al.* [7] reported the significance of PlGF as the best predictor candidate, which is consistent with our results. PlGF displayed an 89% specificity and 65% sensitivity. According to **Zeisler *et al.*[8],** who observed that the sFlt-1:PlGF ratio cut off of 38 had significant predictive value in their development cohort research (five hundred women). Some institutions use PlGF by itself and a ratio of angiogenic biomarkers as clinically useful rule-out testing. In a second validation experiment with an additional 550 women, a sFlt-1:PlGF ratios of 38 or less demonstrated a 99.3% negative predictive value, with 80% sensitivity and 78.3% specificity.**Verlohren *et al.*[9]**found that an angiogenic biomarkers ratio cut off of 38 ruled out PE within one week (NPV was 99.3%) or four weeks (NPV was 94.3%), while ratio values above 38 ruled in PE within four weeks (PPV was more than36%).

Lou *et al.* [10] research supported our findings by demonstrating the importance of the angiogenic biomarker ratio in the diagnosis of PE. With threshold values, ROC curves were created. The angiogenic biomarkersratio was greater in PE patients than in normal controls. For all PE, early onset PE (less than 34 weeks), and late onset PE (more than or equal 34 weeks), the angiogenic biomarkersratio had an AUC of 0.98, 0.99, and 0.91, respectively. The fact that severe PE patients had a higher angiogenic biomarkersratio than mild PE patients further demonstrated its utility in determining the severity of PE.

Stepan *et al.* [11]evaluated the diagnostic performance of PlGF alone and the ratio of angiogenic indicators, using a cut off of angiogenic biomarkers ratio of more than or equal 85 for early-onset PE [<34 weeks] or more than or equal 110 late-onset [≥34 weeks] and less than 36 picogram per milliliter for PlGF, respectively, for the diagnosis of PE. The two assays had comparable sensitivity, but the angiogenic biomarkersratio had a little greater specificity. The calculated AUC for PlGF alone was 0.91 and for the angiogenic biomarkersratio was 0.94. Before compared to after 34 weeks of gestation, both assays performed better. **Lara-Barea *et al.*** [12] reported that higher levels of the (sFlt-1/PIGF) ratio were associated with a 2.7-fold increased risk of developing HDP in women.

Our study also proved that their ability in discrimination between both early and late onset PE. PLGF and sFlt-1 had excellent AUC, (AUC=0.992 and 0.984 respectively) and 95% CI (0.967-1 and 0.945-1 respectively) while, sFlt-1/PLGF ratio had perfect AUC (AUC=1) and 95% CI 1-1, for separating PE patients with an early onset from healthy control groups (<34 weeks) (i.e., diagnosis of early onset PE). It has been noted that the combination of markers enhanced the AUC for the diagnosis of PE with early onset. Table 5

displays the top cut off values and performance traits.Also, PLGF, sFlt-1, sFlt-1/PLGF ratio were conducted for discrimination between late onset PE cases and healthy control groups (≥34 weeks) (i.e. diagnosis of late onset PE). PLGF showed excellent AUCs (AUC=0.999) and 95% CI (0.995-1), while sFlt-1 and sFlt-1/PLGF showed perfect AUCs (AUC=1) and 95% CI (1-1) for both. Best cut off values and performance characteristics are shown in table (6).

Regarding the results ofdiscrimination between early and late onset PE many authors were in agreement with our results:

Verlohren *et al.* [(13)]defined thresholds and reference ranges for the diagnosis of early- and late-onset PE, which are in line with our study. They monitored 1194 pregnancies and calculated risk using the sFlt-1/PLGF ratio. Early onset (less than 34 weeks) and late onset (more than 34 weeks/less than 37 weeks) cut offs were defined separately for each group. The goal was to achieve a sensitivity of more than or equal to 95% at the lower cutoff and a specificity of more than or equal to 95% at the higher cutoff for early-onset PE. A specificity of at least 95% was desired for late-onset PE. PE was strongly suggested by sFlt-1/PLGF ratios of more than or equal 85 (20-33+6 weeks) or more than or equal 110 (34-36+6 weeks). The LR+ was 176, and 13. Additionally, a ratio of less than or equal 33 had a negative LR of for gestational ages of less than 34 and more than or equal 34 weeks, respectively, and did admirably in both gestational age categories for the exclusion of PE. To put it another way, pre-eclampsia was diagnosed with 95% sensitivity and 99% specificity at 34 weeks of gestation when the sFlt-1/PLGF ratio was less than or equal to 33/more than or equal to 85, and with 89% sensitivity and 95% specificity otherwise. when the ratio was less than or equal 33/more than or equal 110 at more than or equal 34 weeks.

Lou *et al.* [(10)]showed that the sFlt-1/PLGF ratio was higher in early-onset PE than in late-onset PE. The average Flt-1/PlGF ratio was greater in PE patients. against a healthy control group at the time immediately before fewer than 34 weeks of pregnancy (early onset). **El-Demerdash *et al.*[(14)]** study, which was identical to ours, early in the second trimester showed that the ratio of angiogenic biomarkers was the most effective method for differentiating pre-eclamptic pregnant women from healthy pregnant women. The best cutoff for our ratio (32.6) offers an 84% diagnostic specificity and a 93.3% diagnostic sensitivity. This value is quite near to the previously reported sFlt-1/PIGF ratio cutoff value of 38.4, which detected pre-eclampsia in the second trimester with an 88.5% diagnostic sensitivity and specificity (24 to 28 weeks of gestation)**[(15)].**

CONCLUSION

PE was predicted and diagnosed using angiogenic biomarkers (sFlt-1, PLGF, and sFlt-1/PLGF ratio). Also, they are valuable for assessing the severity of PE and discrimination between both early and late onset PE, with the aim of providing guidance for their use in management of women with PE.

REFERENCES

1. **Poon L, Shennan A, Hyett J *et al.* (2019):** Erratum to "The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention" [Int J Gynecol Obstet 145 Suppl. 1 (2019) 1–33]. International Journal of Gynecology and Obstetrics, 146(3):390-391.
2. **Jena M, Sharma N, Petitt M *et al.* (2020):** Pathogenesis of preeclampsia and therapeutic approaches targeting the placenta. Biomolecules, 10(6):1-25.
3. **Helmo F, Lopes A, Carneiro A *et al.* (2018):** Angiogenic and antiangiogenic factors in preeclampsia. Pathology - Research and Practice, 214(1):7-14.
4. **Ghafourian M, Mahdavi R, Akbari Jonoush Z *et al.* (2022):** The implications of exosomes in pregnancy: emerging as new diagnostic markers and therapeutics targets. Cell Communication and Signaling, 20(1):1-19.
5. **Than N, Posta M, Györffy D *et al.* (2022):** Early pathways, biomarkers, and four distinct molecular subclasses of preeclampsia: The intersection of clinical, pathological, and high-dimensional biology studies. Placenta, 125:10-19.
6. **Cerdeira A, Agrawal S, Staff A *et al.* (2018):** Angiogenic factors: potential to change clinical practice in pre-eclampsia? BJOG., 125(11):1389-1395.
7. **MacDonald T, Walker S, Hannan N *et al.* (2022):** Clinical tools and biomarkers to predict preeclampsia. EBioMedicine, 75:103780.
8. **Zeisler H, Llurba E, Chantraine F *et al.* (2016):** Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. New England Journal of Medicine, 374(1):13-22.
9. **Verlohren S, Brennecke S, Galindo A *et al.* (2022):** Clinical interpretation and implementation of the sFlt-1/PlGF ratio in the prediction, diagnosis and management of preeclampsia. Pregnancy Hypertension, 27:42-50.
10. **Lou W, Jiang F, Hu J *et al.* (2019):** Maternal serum angiogenic factor sFlt-1 to PlGF ratio in preeclampsia: A useful marker for differential diagnosis and prognosis evaluation in Chinese women. Disease markers, 2019:1-8.
11. **Stepan H, Hund M, Gencay M *et al.* (2016):** A comparison of the diagnostic utility of the sFlt-1/PlGF ratio versus PlGF alone for the detection of preeclampsia/HELLP syndrome. Hypertension in pregnancy, 35(3):295-305.
12. **Lara-Barea A, Sánchez-Lechuga B, Campos-Caro A *et al.* (2022):** Angiogenic imbalance and inflammatory biomarkers in the prediction of hypertension as well as obstetric and perinatal complications in women with gestational diabetes mellitus. Journal of Clinical Medicine, 11(6):1-13.
13. **Verlohren S, Herraiz I, Lapaire O *et al.* (2014):** New gestational phase–specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. Hypertension, 63(2):346-352.
14. **El-Demerdash O, Zaki M, El Maraghy M *et al.* (2018):** PlGF, sFlt-1 and sFlt-1/PlGF ratio: Promising markers for predicting pre-eclampsia. Journal of Gynecological Research and Obstetrics, 4(2):018-023.
15. **Lapaire O, Shennan A, Stepan H. (2010):** The preeclampsia biomarkers soluble fms-like tyrosine kinase-1 and placental growth factor: current knowledge, clinical implications and future application. European Journal of Obstetrics and Gynecology and Reproductive Biology, 151(2):122-129.