The effect of placental angiogenic and anti-angiogenic factors on pregnancy outcome in patients with early onset preeclampsia

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Abstract

Objective: The aim was to evaluate the possible effects of anti-angiogenic factors including soluble endoglin (sEng), placental growth factor (Pgf), and soluble fms-like tyrosine kinase 1 (sFlt-1) in both normotensive pregnant patients and preeclampsia (PE) patients.

Material and Methods: The study was carried out at the Departments of Gynecology and Obstetrics and Biochemistry of Yozgat Bozok University Training and Research Hospital. Eighteen women with PE who were pregnant for at least 20 weeks comprised the study group. The control group consisted of 33 pregnant women with no complications and with similar demographic features. In the study, laboratory parameters, demographic characteristics, sEng, sFlt-1, and Pgf levels, delivery type, APGAR scores of the infants, and birthweight were determined and a comparison was made between the groups.

Results: It was found that the sEng level was significantly lower in the PE group compared to the control group ($p<0.05$). In addition, the Pgf, birthweight, and 1st and 5th-minute APGAR scores were significantly lower in the PE group compared to the control group ($p<0.05$).

Conclusion: The decrease in Pgf may have an effect on the pathogenesis of PE and can be utilized for the determination of PE. (J Turk Ger Gynecol Assoc 2021; 22: 212-6)

Keywords: Preeclampsia, soluble fms-like tyrosine kinase 1, soluble endoglin, placental growth factor

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Introduction

Preeclampsia (PE), which can result in maternal and fetal mortality, is a major complication of pregnancy although its etiology remains unclear (1). PE is characterized by a sudden onset of hypertension and end organ damage in a previously normotensive patient (2). PE complicates 2-8% of pregnancies and if early diagnosis is not established, fatal perinatal and maternal complications may occur. Globally, PE leads to more than fifty thousand maternal deaths a year (3). Therefore, accurate detection of PE is critical and can requires close monitoring of patients with PE.

The balance between angiogenic and anti-angiogenic factors is as important as placental vasculature for a healthy placenta. The pathophysiology of PE might be as follows: An inadequate invasion of syncytiotrophoblasts into spiral arteries in the maternal placental bed leads to the impairment of fetal perfusion and, consequently, ischemia-reperfusion attacks in the placental bed. Then, the release of anti-angiogenic factors into the maternal circulation occurs due to lack of blood supply. Finally, maternal systemic endothelial function deteriorates and systems such as hematologic, neurologic, cardiac, pulmonary, renal, and hepatic tissues become involved (4).

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Despite considerable advances in the management of patients with PE, early prediction of these cases remains a challenge. Assessment of the levels of angiogenic factors, such as placental growth factor (Pgf) or anti-angiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng) could be useful (5,6). However, measurement of these biochemical parameters was reported as unable to aid in diagnosis of PE in a study designed by the National Institute for Health and Care Excellence (7). The present study aimed to detect the early onset PE by measuring levels of Pgf, sEng, and sFlt-1 levels.

Material and Methods

The research was carried out at the Gynecology and Obstetrics Department and Biochemistry Laboratory of Yozgat Bozok University Faculty of Medicine, Turkey. The subjects were recruited from patients referred to our clinic between January 2018 and July 2019. Approval was provided from the Ethical Committee of Yozgat Bozok University Faculty of Medicine. Informed consent was taken from all patients. The present study was funded by Yozgat Bozok University Project Coordination Application and Research Center (6602c-TF/19-247).

To detect an effect size of 0.92 at alpha error of 0.05 and statistical power of 0.95, 50 participants would be required for our study. The clinical studies of the present research included 51 women. The participants were divided into study groups and one control group. Eighteen of these participants comprised the study group by meeting the early onset preclinical criteria (8). In accordance with the literature, the diagnosis of PE was made with the presence of hypertension (systolic blood pressure ≥140 mmHg/diastolic blood pressure ≥90 mmHg) emerging after 20 gestational weeks, accompanied by at least one of the following findings: new-onset proteinuria; renal dysfunction; increased transaminases; arthritis, thrombocytopenia; visual impairment; changes in mental status; epigastric tenderness; fetal growth retardation; and umbilical artery disorder. After the diagnosis of PE, all patients were hospitalized and started on corticosteroid (betamethasone 12 mg/day for two days). Both groups were compared with maternal age, gravidity, parity, gestational week, systolic/diastolic blood pressure, protein levels in spot urine samples, protein levels in 24-hour urine, leucocyte count, hemoglobin level, platelet counts and creatinine, urea, liver function markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH)], sEng, sFlt-1, and Pgf levels. In addition, neonatal parameters such as delivery route, first and fifth minute APGAR score, and neonatal birth weight were compared.

Patients having a pregnancy between 20 and 29 weeks and 6 days were included in the study. The gestational week was calculated according to their last menstrual period or by using ultrasonography. For those in the outpatient clinics, the blood pressure values of the subjects were determined using an adult-type blood pressure monitor. The blood pressure values of the patients in the inpatient clinics were measured using a patient monitor. Following a minimum of 12-hour fasting, a 5 mL venous blood sample was taken at 08.00 a.m. from all the patients for laboratory measurements. In the study group, the proteinuria values were determined in a 24-hour urine protein test.

Fasting blood taken from the patients with PE before the administration of any medication and from normotensive pregnant women was centrifuged within 30 minutes and stored at -80 ºC until analysis. sEng, sFlt1 and Pgf levels were assessed with commercial Elisa kits (Quantikine R&D Systems Europe, United Kingdom) and analyzed in accordance with standard protocols. The study was conducted at the Gynecology and Obstetrics Department and Biochemistry Laboratory of Yozgat Bozok University Faculty of Medicine, Turkey.

Exclusion criteria included a preexisting diagnosis of eclampsia or the presence of HELLP syndrome, multiple pregnancies, presence of malignancy, or psychological disorders. Also, those with a history of preeclampsia, metabolic and hormonal diseases (type 1 and type 2 diabetes mellitus and thyroid diseases), and chronic diseases (gestational hypertension, renal, and hepatic diseases) were not included in the study.

Statistical analysis

The statistical evaluation of the study was carried out using SPSS, version 17.00 (SPSS Inc., Chicago, IL, USA). Continuous variables were examined for normality of distribution using either Kolmogorov-Smirnov or Shapiro-Wilk's test. Data was compared using the Mann-Whitney U test. For categorical variables, the chi-square test was used while the independent sample t-test was utilized for continuous variables showing normal distribution. The receiver operating characteristic (ROC) test was performed to determine the threshold value of the data that can have an effect on preeclampsia. The significance level was set as p-value <0.05.

Results

The number of women who participated in the study was 51. The study group included 18 patients with early onset PE while the control group included 33 patients with no PE diagnosis. All patients in the study group had severe PE and fetal growth restriction was found in five of these (data not shown). The age, gravidity, and parity values of the participants were analyzed and the groups were similar (Table 1). The mean values of arterial blood pressures of the control group were: systolic 105.93 (±10.42) mmHg and diastolic 65.31 (±8.31) mmHg. In the PE group these values were 150.27 (±12.65) and 93.33

(±9.07) mmHg for systolic and diastolic pressures, respectively. The difference was statistically significant (p<0.05).

AST, LDH, and urea levels were significantly higher in the PE group compared to the control group (p<0.05). The mean levels of sEng and Pgf were both significantly lower in the PE group than in the control group (Table 2). sFlt-1 tended to be present at higher concentration in the PE group compared to controls, but this was not significant.

Neonatal parameters, such as delivery route, one and five minute APGAR scores, and neonatal birth weight were shown in Table 3. ROC curves for Pgf, sEng, and sFlt-1 were shown in Figure 1A, B, C. The area under the curve (AUC) for Pgf was 0.983 and the cut-off value was 314.97 pg/mL (sensitivity 93.9%, specificity 94.4%). The AUC for sEng was 0.70 with a cut-off value of 6.87 ng/mL (sensitivity 61.1%, specificity 63.6%). Similarly, the AUC for sFlt-1 was 0.754 with a cut-off value of 754.3 ng/mL (sensitivity 88.9%, specificity 66.7%) (Table 4).

Table 1. Demographic characteristics of the patients

	Study group (n=18)	Control group (n=33)	p-value
Age (year)	30.5±3.68	26.65±5.14	0.790
Gravidity	2.94±1.66	2.40±2.15	0.075
Parity	1.47±1.17	0.71±0.72	0.014
Systolic blood pressure (mmHg)	150.27±12.65	105.93±10.42	<0.001
Diastolic blood pressure (mmHg)	93.33±9.07	65.31±8.31	<0.001
Values are given as mean ± standard deviation			

Table 2. Biochemical parameters of the patients

	PE group (n=18)	Control group (n=33)	p-value
AST (µ/L)	35.66±33.09	16.71±6.31	**<0.001***
ALT (iU/L)	28.88±21.49	15.78±7.65	**0.006***
Urea (mg/dL)	8.88±3.72	6.65±1.87	**0.001***
Creatinine (µmol/L)	0.60±0.09	0.53±0.04	**0.006***
LDH (iU/L)	262.25±108.44	176.07±28.33	**<0.001***
WBC (µ/L)	10,616±3,494	9,981±2,263	0.801
Platelet (/µL)	225,555±77,555	225,781±49,922	0.801
Hb (g/dL)	11.90±1.29	12.00±1.20	0.801
sEng (ng/mL)	5.54±0.68	7.30±0.67	**<0.001***
sFlt-1 (ng/mL)	803.34±52.03	743.71±141.48	**0.044***
Pgf (pg/mL)	87.85±18.96	486.33±102.29	**<0.001***
Values are means ± standard deviation. AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, WBC: White blood cell count, Hb: Hemoglobin concentration, sEng: Soluble endoglin, sFlt-1: Soluble fms-like tyrosine kinase-1, Pgf: Placental growth factor			

Discussion

In this clinical study, the relationship between angiogenic and anti-angiogenic factors, including sEng, Pgf, and SFlt-1, and the outcome of early-onset PE was investigated. Our study has shown that Pgf levels were significantly lower in patients with early-onset PE. However, sFlt-1 levels were similarly distributed in the two groups. Paradoxically, sEng levels were found to be significantly higher in the control group compared to the PE group. Previous studies reported that higher sEng levels in PE group than control group (6-8).

PE is a systemic disease that begins after the 20th week of pregnancy and progresses with hypertension and proteinuria. PE is a leading cause of both maternal-fetal morbidity and mortality but the etiology of the disease is still a dilemma. Both maternal and fetal/placental factors might be responsible for the pathogenesis of PE. Redman devised the two-stage model hypothesis for PE (9). The first of these (stage 1) is the preclinical stage, associated with inadequate placentation, while the second (stage 2) is the clinical staging associated with the maternal syndrome. However, Roberts and Redman (10) claimed that the two-stage model hypothesis was not valid for all PE cases. Afterwards, Palei et al. (11) presented another theory and suggested that defective placentation causes the formation of vasoactive substances (sFlt-1 and sEng) by creating ischemia-reperfusion attacks in the placental bed. Elevation of these anti-angiogenic agents could cause harmful effects in endothelial cells. Therefore, we hypothesized that early detection of these parameters could be useful for early diagnosis of PE.

Angiogenic factors, such as Pgf, and anti-angiogenic factors, such as sFlt-1 and sEng should work in synchrony. It is assumed that maternal Pgf levels decrease, while maternal sFlt-1 and sEng levels increase before clinical preeclampsia is evident (12). In our study, Pgf was found to be low, in accordance with the literature, while sEng were also low, which is in contrast to previous reports. sFlt-1 tended to be higher in the PE group but this was not significant when compared to the control group. This result may be related to the small number of patients and

Table 3. Perinatal outcome of the subjects

	Study group (n=18)	Control group (n=33)	p-value
Mode of delivery			
C/S (%)	28.88±21.49	15.78±7.65	0.006
1 min APGAR	6.48±1.72	6.95±1.87	0.140
5 min APGAR	7.10±2.09	8.03±1.74	0.790
Neonatal weight (g)	2786,56±1058.44	2954.27±1128.33	0.450
C/S: Cesarean section			

Table 4. Cut off, sensitivity and specifity of the Pgf, sEng, and sFlt-1					
Parameters	**AUC[μ]**	**Cut-off**	**Sensitivity (%)**	**Specifity (%)**	**95% CI**
Pgf	0.983	314.97	93.9	94.4	0.955-1.000
sEng	0.700	6.87	61.1	63.6	0.535-0.836
sFlt-1	0.754	754.30	88.9	66.7	0.621-0.887
sEng: Soluble endoglin, sFlt-1: SolublE fms-like tyrosine kinase, Pgf: Placental growth factor, CI: Confidence interval, AUC[μ]: Area under the curve					

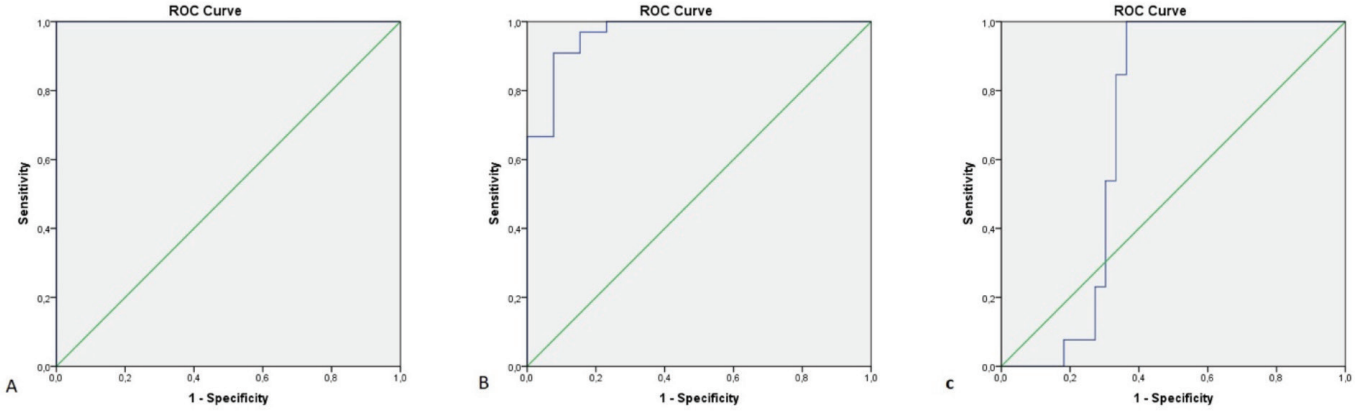

Figure 1. A) ROC curve of Pgf. B) ROC curve of sEng. C) ROC curve of sFlt-1

sEng: Soluble endoglin, sFlt-1: Soluble fms-like tyrosine kinase, Pgf: Placental growth factor, ROC: Receiver operating characteristic

the weeks of gestation. In a study comparing late preeclampsia patients with healthy controls, both sEng and sFlt1 levels were remarkably high in patients with late-onset preeclampsia. However, only sEng may be a useful tool in the determination of the severity of preeclampsia.

Two large studies have been conducted on the measurement of angiogenic and anti-angiogenic factors in PE. One of them was the PARROT trial (13). Pgf alone was evaluated in the PARROT trial. Measurement of Pgf levels was found to be a useful tool to reduce severe maternal complications. However, fetal/neonatal adverse outcomes remained the same. Another large trial was the INSPIRE trial (14) which concluded that these markers alone do not have sufficient use for predicting preeclampsia. Therefore, the sFlt-1/Pgf ratio was measured in the INSPIRE trial. However, it was shown that maternal, fetal, or neonatal outcomes remain still a challenge.

Despite all the previous studies reporting that Pgf, sFlt-1, and sEng levels were important for healthy placentation, there is still debate about their predictive value. Therefore, the present study planned to predict early-onset preeclampsia with these parameters.

Study limitation

The limitations of our study were small patient numbers and heterogeneity of the population.

Conclusion

This study showed that there was a strong association between Pgf and early-onset PE. However, sFlt-1 and sEng were found to be poor in determining early onset preeclampsia.

Ethics Committee Approval: *The study protocol was reviewed and approved by the Ethical Committee of Yozgat Bozok University Faculty of Medicine.*

Informed Consent: *An ethical consent was obtained from the patients.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices - M.K., E.S.Y., M.E.K.; Concept - E.S.Y., D.A.K.; Design - M.K., M.D.Ç.; Data Collection or Processing - T.P., M.D.Ç.; Analysis or Interpretation - E.B., T.O.; Literature Search - M.K., D.A.K.; Writing - M.K., E.B., T.O.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

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References

1. North RA, McCowan LM, Dekker GA, Poston L, Chan EH, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. BMJ 2011; 342: d1875.
2. Yu GZ, Aye CY, Lewandowski AJ, Davis EF, Khoo CP, Newton L, et al. Association of Maternal Antiangiogenic Profile at Birth With Early Postnatal Loss of Microvascular Density in Offspring of Hypertensive Pregnancies. Hypertension 2016; 68: 749-59.
3. Vigil-De Gracia P, Lasso M, Ruiz E, Vega-Malek J, Tem de Mena F, López J; or the HYLA treatment study. Severe hypertension in pregnancy: hydralazine or labetalol. A randomized clinical trial. Eur J Obstet Gynecol 2006; 128: 157-62.
4. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. JAMA 2002; 287: 3183-6.
5. Verlohren S, Stepan H, Dechend R. Angiogenic growth factors in the diagnosis and prediction of pre-eclampsia. Clin Sci (Lond) 2012; 122: 43-52.
6. Stepan H, Schaarschmidt W, Jank A, Verlohren S, Kratzsch J. Use of angiogenic factors (sFlt-1/PlGF ratio) to confirm the diagnosis of preeclampsia in clinical routine: first experience. Z Geburtshilfe Neonatol 2010; 214: 234-8.
7. National Institute for Health and Care Excellence. PlGF-Based Testing To Help Diagnose Suspected Pre-Eclampsia (Triage PlGF Test, Elecsys Immunoassay sFlt-1/PlGF Ratio, DELFIA Xpress PlGF 1-2-3 Test, and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF Plus Kryptor PE Ratio); 2016.
8. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al; International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension 2018; 72: 24-43.
9. Redman C. Pre-eclampsia and the placenta. Placenta 1991; 12: 301-8.
10. Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. Lancet 1993; 341: 1447-51.
11. Palei AC, Spradley FT, Warrington JP, George EM, Granger JP. Pathophysiology of hypertension in pre-eclampsia: a lesson in integrative physiology. Acta Physiol (Oxf) 2013; 208: 224-33.
12. Madazlı R. Gebelikte Tarama ve Öngörü. İstanbul Tıp Kitabevi, 1. Baskı, 2017; p. 197-217.
13. Duhig KE, Myers J, Seed PT, Sparkes J, Lowe J, Hunter RM, et al; PARROT trial group. Placental growth factor testing to assess women with suspected pre-eclampsia: a multicentre, pragmatic, stepped-wedge cluster-randomised controlled trial. Lancet 2019; 393: 1807-18.
14. Cerdeira AS, O'Sullivan J, Ohuma EO, Harrington D, Szafranski P, Black R, et al. Randomized Interventional Study on Prediction of Preeclampsia/Eclampsia in Women With Suspected Preeclampsia: INSPIRE. Hypertension 2019; 74: 983-90.