Original Investigations

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

Kara et al. Early prediction of preeclampsia

Mustafa Kara[1], Ethem Serdar Yalvaç[2], Taylan Onat[2], Emre Başer[2], Melike Demir Çaltekin[2], Demet Aydoğan Kırmızı[2], Müjgan Ercan Karadağ[3]

[1]Department of Obstetrics and Gynecology, Kırşehir Ahi Evran University Faculty of Medicine, Kırşehir, Turkey
[2]Department of Obstetrics and Gynecology, Yozgat Bozok University Faculty of Medicine, Yozgat, Turkey
[3]Department of Medical Biochemistry, Şanlıurfa Harran University Faculty of Medicine, Şanlıurfa, Turkey

Address for Correspondence: Mustafa Kara
Phone: +90 386 280 39 00 e-mail: opdrmustafakara@hotmail.com

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Introduction

Preeclampsia (PE), which can result in maternal and fetal mortality, is the major complication of pregnancy. Its etiology remains unclear. (1). It 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 the early diagnosis could not establish, fatal perinatal and maternal complications could occur. PE leads to more than fifty thousand maternal deaths a year all over the world (3). Therefore, accurate detection of PE could help to close the monitorization of the patients with PE.

The balance between the angiogenic and antiangiogenic factors is essential as much 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 (4). Then, the release of antiangiogenic 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 are involved (5).

Despite considerable advances in the management of patients with PE, the early prediction of these cases remains a challenge. Assessment of the levels of angiogenic factors, such as placental growth factor (Pgf) or antiangiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng) could be useful (6, 7). Contrary, measurement of these biochemical parameters was reported as useless for diagnosis of PE in a study

designed by the National Institute for Health and Care Excellence (8). The present study aimed to detect the early onset PE by measuring the 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, Medical Faculty, Turkey. The subjects were recruited from the patients referred to our clinic between January 2018 and July 2019. Approval was provided from the ethical committee of Yozgat Bozok University Medical Faculty. Informed consent was taken from all of the 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 are required for our study. The clinical studies of the present research included 51 women. The participants were divided into the study groups and one control group. Eighteen of these participants comprised the study group by meeting the early onset preclinical criteria (9). In accordance with the literature, the diagnosis of preeclampsia 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, joint development, thrombocytopenia, visual impairment, mental status changes, epigastric tenderness, fetal growth retardation, and umbilical artery disorder pregnancy visual impairment, mental status changes, epigastric sensitivity, fetal growth retardation, umbilical artery disorder). After the diagnosis of PE, all patients were hospitalized and started on corticosteroid (betamethasone 12 mg/ day for 2 days). Both groups were compared with maternal age, gravidity, parity, gestational week, systolic/diastolic blood pressure levels, protein levels in spot urine, protein levels in 24-hour urine, leucocyte, hemoglobin, platelet counts and creatinine, urea, liver function markers (AST, ALT, LDH), sEng, sFlt-1, and Pgf levels. Also, neonatal parameters such as birth type, 1. and 5. minutes' APGAR score, and neonatal birth weight were compared, too.

Patients having a pregnancy between 20-29 weeks and 6 days were included in the study. The gestational week was measured according to their last menstrual period (LMP) or by using ultrasonography (USG). 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 AM from all the patients for the laboratory parameters. 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 -80º Celsius until analysis. sEng, sFlt1and Pgf levels 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, Medical Faculty, Turkey.

The exclusion criteria of the research included preexisting diagnosed eclampsia or the presence of HELLP syndrome, multiple pregnancies, presence of malignity, or psychological disorders. Also, those with a history of preeclampsia, metabolic and hormonal diseases (type I and type II 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). Continuous variables were examined adopting the analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk's test) for the determination of the presence of a normal distribution. Data normality was evaluated using the Mann-Whitney U test. For categorical variables, the Chi-square test was utilized while the independent sample t-test was utilized for continuous variables showing normal distribution. The receiver operating characteristic (ROC) test was utilized 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 an early onset PE while the control group included 33 patients with no PE diagnosis. All patients in the study group had severe PE, fetal growth restriction was found in 5 of these patients (data not shown). The age, gravidity, and parity values of the participants were analyzed. The differences between the groups were found to be statistically not significant (Table 1). The mean values of arterial blood pressures of the control group were found as systolic 105,93 (±10,42) mmHg, and diastolic 65,31 (±8,31) mmHg; while the systolic and diastolic values of the preeclampsia group were 150,27 (±12,65) 93,33 (±9,07) mmHg, respectively. the difference was statistically significant ($p< 0.05$).

AST, LDH, and urea levels were significantly higher in group 1 than group 2 ($p< 0.05$). The means sEng was 5,54 (±0,68) and 7,30 (±0,67) in group 1 and group 2, respectively, and the difference was statistically significant ($p< 0.05$). Pgf was significantly lower in group 1 than group 2 ($p< 0.05$) (Table 2).

The neonatal parameters such as birth type, 1. and 5. minutes' APGAR score, and neonatal birth weight were shown in Table 3. ROC curves for Pgf, sEng, and sFlt-1 were shown in Figure 1A, B, C. Area under the curve for Pgf was 0.983. The cut-off value for Pgf was 314.97 (sensitivity 93.9%, specificity 94.4%). The area under the curve for sEng was 0.70. The cut-off value for sEng was 6.87 (sensitivity 61.1%, specificity 63.6%). The area under the curve for sFlt-1 was 0.754. The cut-off value for sFlt-1 was 754.3 (sensitivity 88.9%, specificity 66.7%) (Table 4).

Discussion

In this clinical study, the relationship between angiogenic and antiangiogenic factors such as 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 two groups. Paradoxically, sEng levels were found to be significantly higher in group 2 than group 1. The results of this study differ from those of other studies (7-9).

PE is a systemic disease that begins after the 20th week of pregnancy and progresses with hypertension and proteinuria. Despite PE is a leading cause of both maternal-fetal morbidity and mortality, the etiology of the disease is still a dilemma. Both maternal and fetal/placental factors might be responsible for the pathogenesis of PE. Redman reported that the two-stage model hypothesis for PE (10). 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 et al. claimed that the two-stage model hypothesis was not valid for all PE cases (11). Afterwards, Palei et al presented another theory; The researchers have reported that defective placentation causes the formation of vasoactive substances (sFlt-1 and sEng) by creating ischemia-reperfusion attacks in the placental bed (12). Elevation of these antiangiogenic agents could cause a harmful effect in endothelial

cells. Therefore, we thought that the early detection of these parameters could be useful for early diagnosis of PE.
As mentioned above, angiogenic factors such as Pgf and antiangiogenic factors sFlt-1 and sEng should work in harmony. It is assumed that maternal Pgf levels decrease, while maternal sFlt-1 and sEng levels increase before preeclampsia clinical picture emerges (13). In our study, Pgf was found to be low in accordance with the literature, while sEng were found low. The low level of sEng was not compatible with the literature. sFlt-1 was distributed similarly, between the two groups. This result may be related to the small number of patients and the week of gestation. In a study in the literature comparing late preeclampsia patients with healthy controls, both sEng and sFlt1 levels are remarkably high in patients with late-onset preeclampsia; however, only sEng may be a useful tool in the determination of the severity of preeclampsia (14)
Two major trials were conducted about the measurement of angiogenic and antiangiogenic factors in PE. One of them was the PARROT trial (15). Pgf alone was evaluated in the PARROT trial. Pgf was found to be useful to reduce severe maternal complications. However, fetal/neonatal adverse outcomes remained the same. Another big trial was the INSPIRE trial (16). It was claimed that these markers alone do not have sufficient efficiency in predicting preeclampsia. Therefore, the sFlt-1/Pgf ratio was measured in the INSPIRE trial. However, it was shown that maternal, fetal, or neonatal outcomes were not improved by measuring the sFlt-1/Pgf ratio in the INSPIRE trial.
Despite all the previous studies reported 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. 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 weak in predicting early onset preeclampsia.

Ethics committee approval: The study protocol was reviewed and approved by the ethical committee of Yozgat Bozok University Medical Faculty.
Informed Consent: An ethical consent was obtained from the patients.
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.C., Data Collection or Processing: T.O., M.D.C., 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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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 (mm Hg)	150,27±12,65	105,93±10,42	<0,001
Diastolic Blood Pressure (mm Hg)	93,33±9,07	65,31±8,31	<0,001
Values are means ± SD			

Table 2. Biochemical parameters of the patients			
	Study Group (n:18)	**Control Group (n:33)**	**p value**
AST (iu/L)	35,66 (±33,09)	16,71 (±6,31)	**,000***
ALT (iu/L)	28,88 (±21,49)	15,78 (±7,65)	**,006***
Ürea (mg/dl)	8,88 (±3,72)	6,65 (±1,87)	**,001***
Creatinine (µmol/L)	0,60 (±0,09)	0,53 (±0,04)	**,006***
LDH (iu/L)	262,25 (±108,44)	176,07 (±28,33)	**,000***
Wbc (/µL)	10616,66 (±3494,10)	9981,21 (±2263,46)	,801
Platelet (/µL)	225555,55 (±77554,99)	225781,25 (±49921,70)	,801
Hgb (g/dl)	11,90 (±1,29)	12,001 (±1,20)	,801
sEng (ng/ml)	5,54 (±0,68)	7,30 (±0,67)	**,000***
sFlt-1 (ng/ml)	803,34 (±52,03)	743,71 (±141,48)	**,044***
Pgf (pg/ml)	87,85 (±18,96)	486,33 (±102,29)	**,000***
Values are means ± SD. p< 0.05 means statistically significant. AST Aspartate transaminase, ALT Alanine transaminase, LDH Lactate dehydrogenase, Wbc White blood cell, Hgb Hemoglobine, sEng Soluble endogline, sFlt-1 Solubl fms like tyrosine kinase, Pgf Placental growth factor			

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
1min 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)	1586,56±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% Confidence interval**
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 endogline, *sFlt-1* Solubl fms like tyrosine kinase, *Pgf* Placental growth factor					

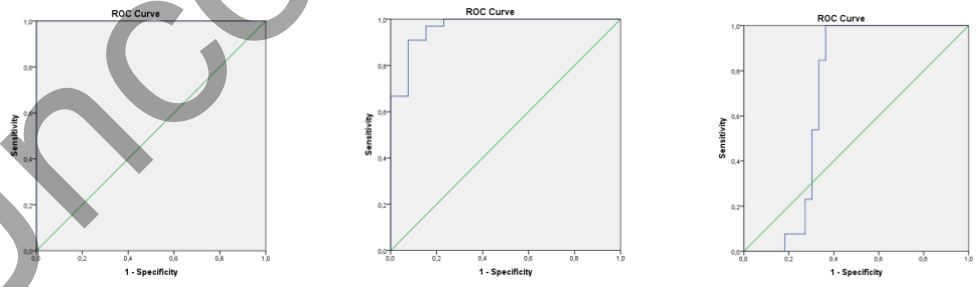

Figure 1A, B, C.. 1A. ROC curve of Pgf. 1B. ROC curve of sEng. 1C. ROC curve of sFlt-1sEng Soluble endogline, sFlt-1 Solubl fms like tyrosine kinase, Pgf Placental growth factor