MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 6924-6931
DOI: 10.12659/MSM.915092

Received: 2019.01.10
Accepted: 2019.04.24
Published: 2019.09.15

Serum Levels of Angiogenic Factors Distinguish Between Women with Preeclampsia and Normotensive Pregnant Women But Not Severity of Preeclampsia in an Obstetric Center in Turkey

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG **Tolga Atakul**

Department of Obstetrics and Gynecology, Adnan Menderes University Faculty of Medicine, Aydın, Turkey

Corresponding Author: Tolga Atakul, e-mail: atakultolga@hotmail.com
Source of support: Departmental sources

Background: This study aimed to compare serum levels of vascular endothelial growth factor (VEGF) and the VEGF receptors, VEGFR-1 and VEGFR-2, free placental growth factor (fPGF), endostatin, and serum pregnancy-associated plasma protein-A (PAPP-A) levels in women with mild and severe preeclampsia and healthy pregnant women.

Material/Methods: A included patients diagnosed with mild preeclampsia (n=32), severe preeclampsia (n=32), and healthy pregnant women (n=24). Serum levels of VEGF-A, VEGFR-1, VEGFR-2, fPGF, endostatin, and PAPP-A levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: In women with mild and severe preeclampsia, the gestation age at birth and birth weight were found to be significantly lower than the control group ($p<0.001$). Serum levels of endostatin, VEGFR-1, and VEGF-A levels were significantly increased in pregnant women with preeclampsia compared with healthy pregnant women ($p<0.001$). Serum levels of PAPP-A, VEGFR-2, and fPGF were significantly higher in healthy pregnant women when compared with women with preeclampsia ($p=0.024$, $p<0.001$, and $p<0.001$, respectively), but there were no significant differences between women with mild and severe preeclampsia.

Conclusions: Reduced serum levels of the angiogenic factors PAPP-A, VEGFR-2, and fPGF distinguished between women with preeclampsia and normotensive pregnant women but did not significantly distinguish between mild and severe preeclampsia.

MeSH Keywords: **Pre-Eclampsia •Pregnancy • Vascular Endothelial Growth Factors**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/915092

2945 3 — 31

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Worldwide, preeclampsia affects between 2–8% of all women in pregnancy and is the main cause of maternal and perinatal mortality and morbidity [1,2]. Preeclampsia accounts for 17–24% of all maternal deaths in developing countries [3,4]. Preeclampsia is a complex clinical condition that is currently believed to have to main causes, inadequate trophoblast invasion and placentation and endothelial damage with impaired angiogenesis [1,2].

In the pathogenesis of preeclampsia, disrupted angiogenic balance and endothelial damage are considered to be associated with the development of clinical symptoms and signs of preeclampsia [3,4]. Vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR), and placental growth factor (PGF) have recently been studied in abnormal angiogenesis and angiogenic balance in preeclampsia [5,6]. The mean plasma and serum levels of soluble VEGFR-1 and soluble fms-like tyrosine kinase-1 (sFlt-1), which are anti-angiogenic in the placenta of patients with preeclampsia have previously been shown to be higher than in healthy pregnant women. Increased sFlt-1 in preeclampsia binds to VEGF and PGF molecules in the circulation and antagonizes their effects [7,8].

Therefore, this study aimed to compare the serum levels of vascular endothelial growth factor (VEGF) and the VEGF receptors, VEGFR-1 and VEGFR-2, free placental growth factor (fPGF), endostatin, and serum pregnancy-associated plasma protein-A (PAPP-A) levels in women with mild and severe preeclampsia and healthy pregnant women.

Material and Methods

Patients

A randomized case-control study included all patients who were diagnosed with mild and severe preeclampsia in the Adnan Menderes University Clinic of Obstetrics and Gynecology and who filled the informed consent forms. A total of 88 patients were included in the study. Thirty-two women were diagnosed with mild preeclampsia, 32 women were diagnosed with severe preeclampsia. Also, 24 healthy pregnant women were included with similar age, and body mass index (BMI). The study protocol was approved by the Ethics Committee of Adnan Menderes University (2017/1203). This study was performed in accordance with the Helsinki Declaration and good clinical practice.

Diagnosis of preeclampsia

The diagnosis of preeclampsia was based on the blood pressure of ≥140\90 mmHg and 300 mg/L of protein (at least +1 on dipstick) in spot urine testing found with two measurements made at least at 6-hourly intervals [9]. The criteria for severe preeclampsia were a blood pressure of ≥160\110 mmHg or oliguria (400 ml/24 hr), impairment of renal function tests, visual impairment, symptoms of cerebral dysfunction, convulsion, headache, epigastric pain, nausea, vomiting, elevation in serum creatinine, thrombocytopenia (<100.000×10^3/mm^3), abnormal peripheral blood smear findings, impaired liver function, pulmonary edema, and cyanosis.

Inclusion and exclusion criteria

All pregnant women with a singleton pregnancy, who were over 18 years of age, >20 weeks' gestation, and who signed an informed consent form were included in the study. Women with multiple pregnancies, with known systemic diseases, pregnant women with hypertension before pregnancy, or with diabetes, and fetal abnormalities, and with cardiac and renal diseases, and urinary tract infections, were excluded from the study.

Obstetric findings

The demographic information (age, parity, height, and weight) and routine laboratory tests of all pregnant women were recorded. The uterine artery resistance at birth, mode of delivery, birth weights, and 1st-minute and 5th-minute APGAR scores of all newborns were recorded. The details of the newborns who were admitted to the newborn intensive care unit (NICU) were recorded. The verbal and written consents of all pregnant women who agreed to participate in the study were recorded. Local Ethics Committee approval was obtained for this study.

Sampling of maternal blood and enzyme-linked immunosorbent assay (ELISA) of serum samples

Blood samples were taken from all patients during labor from the antecubital region of the forearm and were sampled in two sterile vacutainer tubes after the area was wiped with 70% alcohol. Serum samples were divided and placed in three Eppendorf tubes. All samples were centrifuged at 3,000×g for 20 minutes at 4°C and were stored at –85°C.

The serum samples were thawed, and vascular endothelial growth factor-A (VEGF-A), soluble VEGF receptor-1 (sVEGFR-1/sFLt-1), VEGFR-2, free placental growth factor (fPGF), endostatin and pregnancy-associated plasma protein-A (PAPP-A) levels in all maternal samples were analyzed using a commercial VEGF ELISA Kit (Fine Test, Wuhan, China) VEGF Elisa Kit, 2017).

Measurement of serum levels of VEGF-A

Serum VEGF-A levels were measured using a commercial human ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

(Cat. No. E-EL-H0111). The test results were determined with an ELx800 absorbance microplate reader (Biokit, Barcelona, Spain) using a standard curve. The kit detection range was 031.25–2000 pg/ml. The kit performance characteristics were: sensitivity, 18.75 pg/ml, and coefficient of variation (CV%), <10.

Measurement of serum levels of VEGFR1/FLT1

Serum levels of VEGFR1/FLT1 were determined using commercial human ELISA kit (Elabscience Biotechnology Co., Ltd Building 4, Room 401, Guandong Science and Technology Industry Park, Wuhan, China) (Catalogue No. E-EL-H1087). The test results were determined with an ELx800 absorbance microplate reader (Biokit, Barcelona, Spain) using a standard curve. The kit detection range was125–8000 pg/ml. The kit performance characteristics were sensitivity, 75 pg/ml and coefficient of variation (CV%), <10.

Measurement of serum levels of vascular endothelial growth factor receptor 2 (VEGFR2) or KDR

Serum VEGFR2/KDR levels were determined using a commercial human ELISA kit (Elabscience Biotechnology Co., Ltd, Wuhan, China) (Catalogue No: E-EL-H1603). The test results were calculated with an ELx800 absorbance microplate reader (Biokit, Barcelona, Spain) using a standard curve. The kit detection range was 78.13–5000 pg/ml. The kit performance characteristics were sensitivity, 46.88 pg/ml and coefficient of variation (CV%), <10.

Measurement of serum levels of pregnancy-associated plasma protein-A (PAPP-A)

Serum PAPP-A levels determined via commercial human ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) (Catalogue No: E-EL-H0145). The test results were calculated with an ELx800 absorbance microplate reader (Biokit, Barcelona, Spain) using a standard curve. The kit detection range was 0.78–50 ng/ml. The kit performance characteristics were sensitivity, 0.47 ng/ml, and the coefficient of variation (CV%), <10.

Measurement of human endostatin

In serum samples, human endostatin levels were determined using a commercial human ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) (Catalogue No: E-EL-H0063). The test results were calculated with an ELx800 absorbance microplate reader (Biokit, Barcelona, Spain) using a standard curve. The kit detection range was 0.16–10 ng/ml. The kit performance characteristics were sensitivity, 0.1ng/ml, and the coefficient of variation (CV%), <10.

Statistical analysis

SPSS version 25.0 (IBM Corporation, Armonk, NY, USA) was used to analyze the data and variables. The conformity of the data with a normal distribution was evaluated with the Shapiro–Wilk test, and the variance homogeneity with the Levene test. One-way analysis of variance (ANOVA) was determined using the Jonckheere-Terpstra Test. The Kruskal-Wallis test was used to compare the groups according to the quantitative data and Dunn's test. Tukey's test was used for the post hoc analysis. One-way ANOVA was used to compare multiple independent groups according to quantitative data, and the Games-Howell test was used for the post hoc analysis. The Wilcoxon signed rank test was used with the Monte Carlo simulation method for the comparison of the two repetitive measurements of the dependant quantitative variables. The Pearson-Fisher-Freeman-Holton test was used with the Monte Carlo simulation method in the comparison of the categorical variables, and the column rates were compared and expressed according to the Benjamini-Hochberg corrected p-value results. The quantitative variables were shown as the mean ± standard deviation (SD) and median (minimum/maximum) and the categorical variables as n (%) in the tables. The variables were analyzed at 95% confidence interval (CI), and the p-value <0.05 was accepted as significant.

Results

Demographic findings

There were no significant differences between the pregnant women with preeclampsia and without preeclampsia in terms of age, body mass index (BMI), parity, aborted and live birth numbers (p>0.05). In pregnant women diagnosed with preeclampsia, the arterial blood pressures (systolic and diastolic), umbilical artery pulsatility index (UAPI), and protein and creatinine levels in spot urine testing were significantly higher than in healthy pregnant women (p<0.001, p=0.037, and p=0.010, respectively) (Table 1). The gestational age and birth weights were significantly lower in pregnant women diagnosed with preeclampsia (mild and severe) compared with the control group (p<0.001) (Table 1).

Obstetric findings

The APGAR scores at 1 minute and 5 minutes were lower in patients diagnosed with preeclampsia compared with healthy pregnant women (p<0.001), and there was no significant difference between pregnant women diagnosed with mild and severe preeclampsia (p>0.05) (Table 1).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Demograpical data and neonatal APGAR scores in women with normal pregnancy (controls), mild preeclampsia, and severe preeclampsia.

	Control A	Mild preeclampsia B	Severe preeclampsia C	P-value	Pairwise comparison		
	(n=24)	(n=32)	(n=32)		A *vs.* B	A *vs.* C	B *vs.* C
	Mean ±SD	Mean ±SD	Mean ±SD				
Age	28.83±6.75	28.38±5.08	28.84±6.57	0.945	ns	ns	ns
BMI (kg/m^2)	31.13±5.66	30.14±4.48	31.40±5.54	0.607	ns	ns	ns
Birth weight (kg)	3.36±0.27^{B,C}	2.180±0.75	2.082±0.72	**<0.001**	**<0.001**	**<0.001**	0.856
	Median (Min/Max.)	Median (Min/Max.)	Median (Min/Max.)				
Gestational week (week)	38 (36/40)^{B,C}	36 (29/40)	35 (29/38)	**<0.001**	**<0.001**	**<0.001**	0.266
Gravida	2 (1/5)	1 (1/5)	1.5 (1/6)	0.384	ns	ns	ns
Parity	0 (0/4)	0 (0/4)	0 (0/4)	0.647	ns	ns	ns
Abortion	0 (0/1)	0 (0/2)	0 (0/2)	0.483	ns	ns	ns
Live	0 (0/3)	0 (0/3)	0 (0/3)	0.421	ns	ns	ns
Diagnosis gestational (week)	33.8 (28.7/38.5)	34 (29/39)	34.45 (28.7/38)	0.444	ns	ns	ns
Max systolic	107 (91/123)^{B,C}	140 (130/150)^{C}	160 (140/190)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Max diastolic	73 (63/85)^{B,C}	90 (80/100)^{C}	100 (80/110)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAPI	0.895 (0.64/1.23)^{C}	0.99 (0.69/2.5)	1.2 (0.72/2.4)	**0.037**	0.056	**0.001**	0.224
Urinary pro/cre ratio	0.36 (0.11/13.25)^{B,C}	1,25 (0.1/18)^{C}	5.075 (0.33/22)	**0.010**	**0.015**	**<0.001**	**0.006**
APGAR 1. dk.	8 (7/9)^{B,C}	7 (0/9)	6 (3/9)	**<0.001**	**<0.001**	**<0.001**	0.999
APGAR 5. dk.	10 (9/10)^{B,C}	8 (0/10)	8 (6/10)	**<0.001**	**<0.001**	**<0.001**	0.999

One-way analysis of variance (ANOVA) (Robust Statistic: Brown-Forsythe); Post hoc test: Games Howell, Jonckheere-Terpstra Test (Monte Carlo); Post hoc test: Dunn's test, Wilcoxon signed ranks test (Monte Carlo); SD – standard deviation; Min – minimum; Max – maximum; A – significant compared with the control group; B – significant compared with the mild preeclampsia group; C – significant compared with the severe preeclampsia group; UAPI – umbilical artery pulsatility index; min – minute.

Almost 50% of the healthy pregnant women had a cesarean section, but the cesarean section rate was significantly higher in pregnant women with severe preeclampsia (81.3%) compared with women with mild preeclampsia and healthy pregnant women (Table 2). Births with intrauterine growth restriction (IUGR) were found in half of the pregnant women diagnosed with preeclampsia (p<0.001), but there was no difference between the rates of birth with IUGR in the pregnant women diagnosed with mild and severe preeclampsia (p>0.05).

Laboratory results

There was no significant difference between pregnant women in terms of blood urea nitrogen (BUN), creatinine, hemoglobin (Hb), white blood cell (WBC) count, platelets (PLT), aspartate transaminase (AST) and alanine transaminase (ALT) results (p>0.05). The uric acid levels for pregnant women diagnosed with mild and severe preeclampsia were significantly higher than the control group (p<0.001) (Table 3).

The levels of endostatin, vascular endothelial growth factor-A (VEGF-A), and the VEGF receptor-1) were significantly higher in pregnant women diagnosed with preeclampsia compared

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Obstetric results.

		Controls A (n=24) n (%)	Mild preeclampsia B (n=32) n (%)	Severe preeclampsia C (n=32) n (%)	P-value
Mode of delivery	NSVD	12 (50)[C]	14 (43.8)[C]	6 (18.8)	**0.022**
	C/S	12 (50)	18 (56.3)	26 (81.3)[A,B]	
IUGR	Absent	24 (100)[B,C]	15 (46.9)	17 (53.1)	**<0.001**
	Present	0 (0)	17 (53.1)[A]	15 (46.9)[A]	
Uterine resistance increase	Absent	24 (100)[B,C]	11 (34.4)	9 (28.1)	**<0.001**
	Present	0 (0)	21 (65.6)[A]	23 (71.9)[A]	
NICU admission	Absent	24 (100)[B,C]	17 (53.1)	11 (34.4)	**<0.001**
	Present	0 (0)	15 (46.9)[A]	21 (65.6)[A]	

Fisher Freeman Halton test (Monte Carlo); A – significant compared with the control group; B – significant compared with the mild preeclampsia group; C – significant compared with the severe preeclampsia group; NSVD – normal spontaneous vaginal delivery; C/S – cesarean section; IUGR – intrauterine growth restriction; NICU – Nwborn Intensive Care Unit.

with healthy pregnant women (p<0.001). However, the pregnancy-associated plasma protein-A (PAPP-A), VEGFR-2, and free placental growth factor (fPGF) levels were higher in healthy pregnant women (p=0.024, p<0.001, and p<0.001, respectively). There was no significant difference between women with mild and severe preeclampsia in terms of these biomarkers (p>0.05) (Table 3).

Discussion

In a healthy pregnancy, the placental cytotrophoblast cells invade the uterine wall and high-resistance uterine spiral arteries and arterioles form a low-resistance vascular bed [10]. This angiogenic remodeling is abnormal in women with preeclampsia, and placental ischemia develops [11]. In placental development, vascular endothelial growth factor (VEGF) and the VEGF receptors, VEGFR-1 and VEGFR-2, and free placental growth factor (fPGF), which are members of VEGF family, have been studied and are believed to be involved in angiogenic balance in placentation [12–14].

This study aimed to measure serum levels of factors involved in the development and regulation of the placental vasculature in maternal serum obtained at birth. Women with mild and severe preeclampsia were compared with healthy pregnant women. The findings were consistent with those reported by previous studies [15,16]. Serum levels of endostatin, VEGFR-1, and VEGF-A were significantly increased in women with preeclampsia, whereas free placental growth factor (fPGF) and serum pregnancy-associated plasma protein-A (PAPP-A) levels were significantly higher in healthy pregnant women [15,16].

VEGF binds to its receptors, VEGFR-1 (sFlt1) or VEGFR-2, whereas fPGF binds specifically to the sFlt-1 receptor [7]. As a result of placental dysfunction, a significant increase occurs in the production of sVEGFR-1 (sFlt-1), or soluble VEGFR-1. Previous studies have shown that sFlt-1 was significantly increased in the development of preeclampsia and bound to VEGF and fPGF in the circulation, reducing the free levels of these molecules in the plasma and created an anti-angiogenic effect [5,7,17].

Geva et al. reported that women with preeclampsia who gave birth to infants with intrauterine growth restriction (IUGR), had increased serum levels of VEGF-A, VEGFR-1, and sVEGFR-1 levels, but there was no change in the fPGF, VEGFR-2, and (neuropilin-1) NRP-1 levels [16]. The increase in VEGF, VEGFR-1, and sVEGFR-1 levels in pregnant women with preeclampsia were considered to be associated with placental hypoxia [16,17]. Bahlman et al. showed that the diagnostic sensitivity of the combined use of the sFlt-1/PGF ratio and uterine artery pulsatility index (UAPI) was 74% for the diagnosis of preeclampsia, and the sensitivity was 83.7%, and specificity was 68.1% when the cut-off value was taken as 5424 pg/ml for the sFlt-1 [18]. High sFlt-1 levels developed before the clinical symptoms and the sFlt-1 levels were associated with the time of onset of clinically evident preeclampsia, and partly with the severity of preeclampsia [18]. It was previously shown that sFlt-1 levels were

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. Laboratory results.

	Control A		Mild preeclampsia B		Severe preeclampsia C		P-value	Pairwise comparison		
	(n=24)		(n=32)		(n=32)			A *vs.* B	A *vs.* C	B *vs.* C
	Mean ±SD		Mean ±SD		Mean ±SD					
BUN (mg/dl)	11.36±2.37		10.71±3.26		10.89±3.47		0.726	ns	ns	ns
Hb (g/dl)	11.34±1.17		11.47±1.64		11.37±1.45		0.932	ns	ns	ns
	Median (Min/Max.)		Median (Min/Max.)		Median (Min/Max.)					
Uric acid (mg/dL)	4.3	(3.0/5.8)[B,C]	5.6	(2.9/8.9)	6.5	(2.6/9.6)	**<0.001**	**0.001**	**<0.001**	0.052
WBC (10^3/mm^3)	10.4	(7.65/14.7)	10.8	(7.650/23)	11.05	(5.71/24)	0.342	ns	ns	ns
PLT (10^3/mm^3)	220	(138/395)	168	(125/491)	187.5	(60/433)	0.362	ns	ns	ns
AST (U/L)	18.0	(9/53)	15.5	(9/53)	15	(9/52)	0.265	ns	ns	ns
ALT(U/L)	16,0	(6/58)	15.5	(6/58)	14.5	(6/43)	0.271	ns	ns	ns
LDH (IU/L)	227	(124/280)	219.5	(124/380)	255	(165/379)	0.745	ns	ns	ns
Creatinine (mg/dl)	0.7	(0.4/0.8)	0.6	(0.4/0.8)	0.7	(0.5/1)	0.338	ns	ns	ns
Endostatin (ng/ml) X Dil.F	17	(13/89)[B,C]	41	(14/220)	35	(25/180)	**<0.001**	**<0.001**	**<0.001**	0.385
PAPP-A (ng/ml)	32.1	(3.9/121.4)[B,C]	25.9	(2.9/67.4)	16.3	(5.9/31.7)	**0.024**	**0.037**	**0.002**	0.235
sVEGFR-1 (ng/ml)	1.57	(1.35/4.4)[B,C]	2.82	(1.52/7.97)	3.57	(1.39/18.15)	**<0.001**	**<0.001**	**<0.001**	0.901
sVEGFR-2 (ng/ml)	10.04	(8.63/37.92)[B,C]	6.9	(4.99/14.5)	7.03	(3.2/9.57)	**<0.001**	**<0.001**	**0.007**	0.108
VEGF-A (ng/ml)	10.16	(5.41/28.21)[B,C]	74.93	(10.12/398.45)	54.58	(22.75/198.38)	**<0.001**	**<0.001**	**<0.001**	0.303
fPGF (ng/ml)	532	(133.2/1380)[B,C]	349.6	(66.1/900.1)	237.2	(2.6/716.2)	**<0.001**	**<0.001**	**<0.001**	0.076

One-way analysis of variance (ANOVA) (Robust Statistic: Brown-Forsythe); Post hoc test: Games Howell, Jonckheere-Terpstra Test (Monte Carlo); Post hoc test: Dunn's test, Wilcoxon signed ranks test (Monte Carlo); SD – standard deviation; Min – minimum; Max – maximum; A – significant compared with the control group; B – significant compared with the mild preeclampsia group; C – significant compared with the severe preeclampsia group; WBC – white blood cells; VEGF – vascular endothelial growth factor; sVEGFR – soluble vascular endothelial growth factor receptor; PAPP-A – pregnancy-associated plasma protein-A; fPGF – free placental growth factor.

higher in early-onset cases of preeclampsia [15]. Also, in a recent animal experimental study, in a model of hypertension associated with proteinuria, impaired renal function, thrombocytopenia, abnormal liver function and epigastric pain, with hemolysis-elevated liver enzymes-low platelet count (HELLP) syndrome was induced following VEGF-associated reduction in endothelial nitric oxide synthase (eNOS) production [19].

Soluble placental growth factor (sPGF) is a proangiogenic protein secreted from the placenta, which peaks in the 30th gestational week and levels then reduce until the end of pregnancy [6]. Also, sPGFplays a role in angiogenesis, vasculogenesis, and embryogenesis during pregnancy [6]. Reduction in free PGF (fPGF) is found in the serum in late pregnancy due to binding of fPGF to sFlt-1 [6]. However, previous studies have shown that there was no change, or there was an increase in the fPGF levels in placental tissues in preeclampsia [15,20]. Previous studies have shown that low levels of fPGF in pregnant women diagnosed with preeclampsia were secondary to the binding of the fPGF molecules by sFlt-1 that were increased in the circulation secondary to dysfunction of the placenta [20,21]. In the present study, serum levels of fPGF were found to be higher in healthy pregnant women, which is in keeping with previously published findings.

PAPP-A is a glycoprotein secreted in the placenta, and it has been used for more than three decades as an important

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

biochemical indicator in pregnancy [21]. Maternal plasma concentrations of PAPP-A increase during pregnancy [22]. In the first trimester of the pregnancy, PAPP-A is used in combination with the gonadotropin, β-human chorionic gonadotropin (β-hCG), and nuchal translucency (NT) thickness for screening for trisomy 21, 13, and 18 [22]. Previously published studies have reported the measurement of PAPP-A levels at different stages of pregnancy and to predict pregnancy outcomes [23]. Reduced PAPP-A levels in the first trimester of pregnancy were found to be associated with an increased risk of preeclampsia, intrauterine growth restriction (IUGR), small for gestational age (SGA), and preterm birth [23]. Also, measurement of serum PAPP-A combined with the uterine artery pulsatility index (UAPI) is a strong predictive (70%) biochemical indicator for the diagnosis of preeclampsia [23]. Although previous studies showed that the plasma PAPP-A concentrations increased in pregnancies complicated by preeclampsia and HELLP, the association with the severity of preeclampsia could not be demonstrated [24–27]. Although there is no clear consensus about the use of measurement of PAPP-A level in the diagnosis and follow-up of preeclampsia, its combined use with other serum indicators has been recommended [28].

Previous studies have also shown that the serum levels of PAPP-A in women with preeclampsia in the second trimester were lower than those in healthy pregnant women [8,21,29]. However, serum levels of PAPP-A in women with preeclampsia towards the end of the third trimester reached a higher level compared with healthy pregnant women [21]. Decreased PAPP-A levels in the first trimester are believed to play an important role in the development of preeclampsia. In the present study, the levels of maternal serum PAPP-A obtained at birth were significantly increased in healthy pregnant women. The findings from previous studies supported these results. However, the PAPP-A levels obtained at birth were not associated with the severity of preeclampsia.

Endostatin is an active component of the collagen XVII and is an inhibitor of angiogenesis by blocking VEGF signaling via the VEGFR-2 receptor (or KDR). Endostatin production, together with other anti-angiogenic factors, triggers the development of preeclampsia. However, few previous studies have evaluated the effect of endostatin alone or in combination with other anti-angiogenic molecules on the development of preeclampsia. Endostatin levels have been shown to increase, particularly in the 34th to 37th weeks in pregnant women with preeclampsia [30]. In the present study, endostatin levels were significantly increased in pregnant women diagnosed with preeclampsia compared with healthy pregnant women, which was supported by previous studies.

Infants with APGAR scores (cardiac apex beat, ventilatory effort, color, muscular tonus, response to a stimulus, color) between 7–10, taken in the first and fifth minutes after delivery, are usually healthy infants who do not require further follow-up. However, previous studies have shown that preeclampsia resulted in low neonatal APGAR scores, intrauterine death, low birth weight, intrauterine growth restriction (IUGR), and increased admission to the neonatal intensive care unit (NICU) [31]. In the present study, infants with the lowest average APGAR score, birth weight, and most severe degrees of IUGR were from pregnant women with preeclampsia, and there was a need for admission to the NICU in 65% of these infants.

This study had several limitations. There was a small number of study participants, and the study was conducted at a single center, which could have introduced bias. There were differences between the number of weeks of gestation of the pregnant women included in the study, which resulted in difficulty in the comparison of the birth weights, laboratory results, and clinical findings in the newborns. Differences in the gestational age and birth weights may have been associated with the high rate of early cesarean section in pregnant women diagnosed with preeclampsia compared with healthy pregnant women.

Conclusions

This study aimed to compare serum levels of vascular endothelial growth factor (VEGF) and the VEGF receptors, VEGFR-1 and VEGFR-2, free placental growth factor (fPGF), endostatin, and serum pregnancy-associated plasma protein-A (PAPP-A) levels in women with mild and severe preeclampsia and healthy pregnant women. Reduced serum levels of the angiogenic factors PAPP-A, VEGFR-2, and fPGF distinguished between women with preeclampsia and normotensive pregnant women but did not significantly differentiate between mild and severe preeclampsia. These findings were from a study in a single center, and there is a need for large-scale multicenter studies controlled studied to further investigate the role of serum markers of angiogenesis in preeclampsia.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Pratt A, Da Silva Costa F et al: Placenta-derived angiogenic proteins and their contribution to the pathogenesis of preeclampsia. Angiogenesis, 2015; 18(2): 115–23
2. Majedand BH, Khalil RA: Molecular mechanisms regulating the vascular prostacyclin pathways and their adaptation during pregnancy and in the newborn. Pharmacol Rev, 2012; 64(3): 540–82
3. Torry DS, Wang HS, Wang TH et al: Preeclampsia is associated with reduced serum levels of placenta growth factor. Am J Obstet Gynecol, 1998; 179(6 Pt 1): 1539–44
4. Maynard SE, Min JY, Merchan J et al: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest, 2003; 111(5): 649–58
5. Chaiworapongsa T, Romero R, Kim YM et al: Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J Matern Fetal Neonatal Med, 2005; 17(1): 3–18
6. Rădulescu C, Bacârea A, Huanu A et al: Placental growth factor, soluble fms-Like tyrosine kinase 1, soluble endoglin, IL-6, and IL-16 as biomarkers in preeclampsia. Mediators Inflamm, 2016; 2016: 3027363
7. Levine RJ, Maynard SE, Qian C et al: Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med, 2004; 350(7): 672–83
8. Anderson UD, Olsson MG, Kristensen KH et aL: Review: Biochemical markers to predict preeclampsia. Placenta, 2012; 33(Suppl.): S42–47
9. Roberts JM, August PA, Bakris G et al: Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy. Obstet Gynecol, 2013; 122(5): 1122–31
10. Sood R, Kalloway S, Mast AE et al: Fetomaternal cross talk in the placental vascular bed: control of coagulation by trophoblast cells. Blood, 2006; 107(8): 3173–80
11. Hariharan N, Shoemaker A, Wagner S: Pathophysiology of hypertension in preeclampsia. Microvasc Res, 2017; 109: 34–37
12. Demir R, Seval Y, Huppertz B: Vasculogenesis and angiogenesis in the early human placenta. Acta Histochem, 2007; 109(4): 257–65
13. Mundim GJ, Paschoini MC, Araujo Júnior E et al: Assessment of angiogenesis modulators in pregnant women with pre-eclampsia: A case-control study. Arch Gynecol Obstet, 2016; 293(2): 369–75
14. Shenoy V, Kanasaki K, Kalluri R: Pre-eclampsia: Connecting angiogenic and metabolic pathways. Trends Endocrinol Metab, 2010; 21(9): 529–36
15. Hoeller A, Ehrlich L, Golic M et al: Placental expression of sFlt-1 and PlGF in early preeclampsia *vs.* early IUGR *vs.* age-matched healthy pregnancies. Hypertens Pregnancy, 2017; 36(2): 151–60
16. Geva E, Ginzinger DG, Zaloudek CJ et al: Human placental vascular development: Vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2. J Clin Endocrinol Metab, 2002; 87(9): 4213–24
17. Chaiworapongsa T, Romero R, Espinoza J et al: Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. Am J Obstet Gynecol, 2004; 190(6): 1541–47
18. Bahlmann F, Al Naimi A: Using the angiogenic factors sFlt-1 and PlGF with Doppler ultrasound of the uterine artery for confirming preeclampsia. Arch Gynecol Obstet, 2016; 294(6): 1133–39
19. Oe Y, Ko M, Fushima T et al: Hepatic dysfunction and thrombocytopenia induced by excess sFlt1 in mice lacking endothelial nitric oxide synthase. Sci Rep, 2018; 8(1): 102
20. Khaliq A, Dunk C, Jiang J et al: Hypoxia down-regulates placenta growth factor, whereas fetal growth restriction up-regulates placenta growth factor expression: Molecular evidence for "placental hyperoxia" in intrauterine growth restriction. Lab Invest, 1999; 79(2): 151–70
21. Westergaard JG, Teisner B, Grudzinskas JG: Serum PAPP-A in normal pregnancy: Relationship to fetal and maternal characteristics. Arch Gynecol, 1983; 233(3): 211–15
22. Kagan KO, Wright D, Valencia C et al: Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. Hum Reprod, 2008; 23(9): 1968–75
23. Patil M, Panchanadikar TM, Wagh G: Variation of PAPP-A level in the first trimester of pregnancy and its clinical outcome. J Obstet Gynaecol India, 2014; 64(2): 116–19
24. Ranta JK, Raatikainen K, Romppanen J et al: Decreased PAPP-A is associated with preeclampsia, premature delivery and small for gestational age infants but not with placental abruption. Eur J Obstet Gynecol Reprod Biol, 2011; 157(1): 48–52
25. Odibo AO, Zhong Y, Longtine M et al: First-trimester serum analytes, biophysical tests and the association with pathological morphometry in the placenta of pregnancies with preeclampsia and fetal growth restriction. Placenta, 2011; 32(4): 333–38
26. Spencer K, Yu CK, Cowans NJ et al: Prediction of pregnancy complications by first-trimester maternal serum PAPP-A and free beta-hCG and with second-trimester uterine artery Doppler. Prenat Diagn, 2005; 25(10): 949–53
27. Smith GC, Stenhouse EJ, Crossley JA et aLl: Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab, 2002; 87(4): 1762–67
28. Cuckle HS: Screening for pre-eclampsia – lessons from aneuploidy screening. Placenta, 2011; 32(Suppl.): S42–48
29. Kirkegaard I, Uldbjerg N, Oxvig C: Biology of pregnancy-associated plasma protein-A in relation to prenatal diagnostics: Aan overview. Acta Obstet Gynecol Scand, 2010; 89(9): 1118–25
30. Hirtenlehner K, Pollheimer J, Lichtenberger C et al: Elevated serum concentrations of the angiogenesis inhibitor endostatin in preeclamptic women. J Soc Gynecol Investig, 2003; 10(7): 412–17
31. Ayaz A, Muhammad T, Hussain SA, Habib S: Neonatal outcome in preeclamptic patients. J Ayub Med Coll Abbottabad, 2009; 21(2): 53–55

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]