Maternal soluble vascular cytoplasmic adhesion molecule-1 and fibronectin levels in early- and late-onset preeclamptic pregnancies

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Summary

Objective: The purpose of this study was to investigate maternal plasma soluble vascular cytoplasmic adhesion molecule-1 (sVCAM-1) and fibronectin levels in the patients with early-onset preeclampsia (EOP) and late-onset preeclampsia (LOP) and also to determine whether different mechanisms are involved in these two forms of disorders. *Material and Methods:* The authors performed a case control study consisting of randomly selected 80 healthy pregnant women (group 1= control group) and 80 preeclamptic women (group 2= defined study group). Study group consisted of 43 patients with EOP and 37 patients with LOP. sVCAM-1 and fibronectin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) and the findings were compared between the groups. *Results:* The mean levels of sVCAM-1 and fibronectin were significantly higher in the LOP group than those in the normotensive group (p = 0.043 and 0.010 respectively). Markers were significantly different between the two hypertensive groups of pregnancy. The EOP group had a higher level of sVCAM-1 and fibronectin concentration than the LOP group (p = 0.01, for both markers). There was a positive correlation both between the values of plasma fibronectin and the systolic- diastolic blood pressure measurements (r:0.43 and 0.44, respectively), and between sVCAM-1 and the systolic/diastolic blood pressure measurements (r = 0.54 and 0.64, respectively). *Conclusion:* Increased plasma levels of fibronectin and sVCAM-1 were found in the preeclamptic patients, especially in those with early-onset preeclampsia. These markers might be related to the pathogenesis of different types of preeclampsia.

Key words: Soluble vascular cytoplasmic adhesion molecule-1; Fibronectin; Early- and late-onset preeclampsia.

Introduction

Preeclampsia is a heterogeneous human pregnancy syndrome that affects several organ systems [1]. It has been characterized by some investigators as two different diseases: early onset preeclampsia (EOP) and late onset preeclampsia (LOP)- on the basis of gestational age [2-3]. EOP is usually defined as preeclampsia that develops before 34 weeks of gestation, while LOP develops at or after 34 weeks of gestation. Although the diagnostic criteria are the same in each of these phenotypic variants of preeclampsia, they are characterized by different clinical features and are associated with different maternal and fetal outcomes [2]. Gestational age at the onset of the disease is not considered as a criterion for the diagnosis or subclassification of preeclampsia [3].

Preeclampsia is proposed to occur in two stages. Stage 1 comprises reduced placental perfusion, which is postulated as the root cause that leads to Stage 2, namely the maternal syndrome [4]. It is believed that placental ischaemia during Stage 1 may lead to placental production factors, one of which is cytokines that cause the activation of adhesion molecules [5, 6]. The cell adhesion molecules play a role in leukocyte-endothelial interaction and diapedesis [5, 6]. The basic processes of leukocytes are to phagocyte the useful factors and to produce toxic factors. These toxic factors (elastase, myeloperoxidase) injure the endothelium [6, 7] and dysfunctional endothelium leads to the clinical syndrome of hypertension and proteinuria [8].

Adhesion molecules are divided into groups according to their structures: selectins, integrins, cadherins, and members of the immunoglobulin gene superfamily. Besides these groups, there are some molecules performing the same functions as adhesion molecules like fibronectin [9, 10]. sVCAM-1 is a cell adhesion molecule and a member of the immunoglobulin superfamily. sVCAM-1 is important for recruiting leukocytes to the sites of inflammation because it mediates the adhesion of lymphocytes, monocytes, and eosinophils to endothelium [11].

Increased levels of sVCAM-1 and fibronectin in the patients with preeclampsia could be indicative of endothelial cell activation and the soluble adhesion molecules in plasma should reflect the concentration of membrane-bound adhesion molecules on the endothelium. Thus, in this study the authors aimed to evaluate the role of sVCAM- 1 and fibronectin in the patients with EOP and LOP.

Material and Methods

This study was conducted as a case-control study in prospective cross-sectional cohort type between March 2008 and March 2009. Eighty normotensive patients indicated as healthy pregnant according to the results of the examinations were included in this

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Table 1. — *Clinical characteristics of study and control groups.*

	EOP (n=43)	LOP (n=37)	Normotensive controls (n=80)
Maternal age (years)	29.39±5.91	30.12±1.92	28.00±5.84
Gravidity	1.42±1.38	1.43±1.56	0.93±1.18
GA	25.49±3.72**	34.12±1.12*	37.65±2.50
Birth weight (g)	1819.32±800.4**	2467.44±996.09*	3209.38±648.47
SBP (mmHg)	160.12±15.12**	150.90±13.01*	110.87±10.34
DBP (mmHg)	110.14±14.13**	102.60±10.81*	67.87±8.22

Data are presented as mean ± standard deviation (SD).
*Significant difference in comparison with normotensive and late-onset preeclamptic groups ($p < 0.05$).
**Significant difference in comparison with early-onset and late-onset preeclamptic groups ($p < 0.05$).
GA: Gestational age at delivery; SBP: Systolic blood pressure;
DBP: Diastolic blood pressure.

Table 2. — *Biochemical parameters of study and control groups.*

	EOP (n=43)	LOP (n=37)	Normotensive controls (n=80)
sVCAM-1 (ng/ml)	87.27±38.11**	50.59±33.22*	45.03±33.91
Fibronectin (ng/ml)	30.22±8.29**	20.33±5.11*	12.97±10.83
AST (U/L)	70.44±30.33**	34.59±38.72*	18.32±5.37
ALT(U/L)	76.33±23.22**	33.71±31.75*	18.16±5.05
BUN (mg/dl)	10.81± 3.21	10.12±2.33	10.63±3.21
Creatinin (mg/dl)	0.86±0.40	0.78±0.80	0.77±0.39
Hematocrite (%)	34.45±4.67	33.34±4.34	32.94±4.25

*Significant difference in comparison with normotensive and late-onset preeclamptic groups ($p < 0.05$).
**Significant difference in comparison with early-onset and late-onset preeclamptic groups ($p < 0.05$).

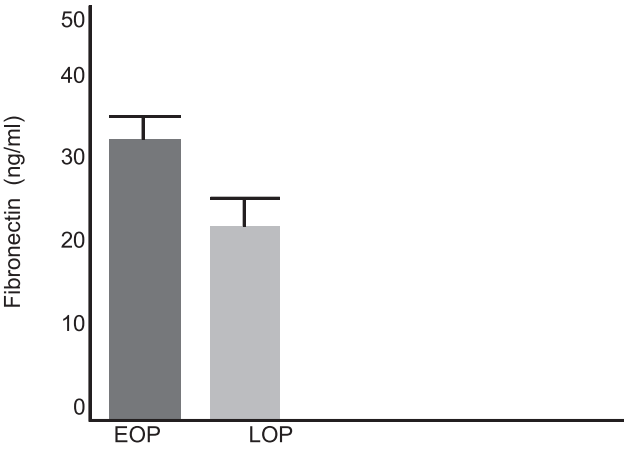

Figure 1. — Serum concentrations of fibronectin in EOP and LOP ($p = 0.01$)

study as control group (group 1) and 80 patients with preeclampsia who were hospitalized in the Obstetrics Clinic of Medical Faculty at Çukurova University were included as study group (group 2). Preeclamptic patients were divided into two groups according to the onset of the preeclampsia: early-onset preeeclamptics (< 34 weeks gestation) (n=43) and LOPs (≥ 34 weeks gestation) (n=37). At the stage of sampling for blood analysis, medical consent was taken from each case included in the study in accordance with the ethical issues in the Declaration of Helsinki. Furthermore, ethical approval of the Ethics Committee of Çukurova University was obtained. The women who gave written informed consent were recruited in this study. The cases of the study and control groups were chosen from the patients whose pregnancies were in the 20th and 41st weeks. While indicating gestational age, the dates of the last menstruation and obstetrical ultrasonographic examination were taken into consideration. All of the cases were chosen from the pregnants who had no histories about hypertension or any diseases affecting energy metabolism and drug use, smoking, singleton, diabetes, and any autoimmune systemic diseases. Patients whose tension arterial rates were over 140/90 mmHg in two measuring six hours apart and protein losses were over 300 mg/l in urine in 24 hours or who had one positive proteinuria in spot urine were accepted as preeclamptic. Control group consisted of the pregnants who had no histories about systemic diseases and drug use, smoking, normotension, and proteinuria. Demographic data and histories, complete blood count, and complete urine analysis were checked for all pregnants included in the study. Also, serum blood urea nitrogen, creatinine, AST, ALT, and LDH values were evaluated. In addition to this, the values of total protein were controlled by collecting a 24-hour urine sample from the preeclamptic group. Venous blood samples of all pregnants from antecubital zone were analyzed. After blood samples taken from fibronectin and sVCAM- 1 antecubital vein under sterile conditions were centrifuged for five minutes in 3,500 cycles, their plasma was taken and kept in -70ºC in laboratory until analyses were begun. Serum levels of fibronectin and sVCAM-1 were measured by commercial enzyme-linked immunosorbent assay (ELISA) assay according to the manufacture's instructions.

All values were expressed as means ± SD (standard error of mean). Statistical tests were performed using SPSS (Statistical Package for Social Sciences) version 15.0. Variations over statistically significant results and rates between the groups were indicated by using Whitney U, chi-square, and Spearman correlation tests. A p-value of < 0.05 was considered statistically significant.

Results

A significant variation was not indicated in terms of age and gravid between preeclamptic and normotensive pregnants. Gestational weeks at delivery and birth weight of the newborns were significantly lower in the LOP group than in the normotensive group and were significantly lower in the EOP group than in the LOP group ($p < 0.05$). As expected, blood pressure measurements were significantly higher in the LOP group than in the normotensive group ($p < 0.05$) and blood pressure measurements were significantly higher in the EOP group than in the LOP group ($p < 0.05$, Table 1).

The serum concentrations of sVCAM- 1, fibronectin, AST, and ALT were significantly higher in the LOP group than in the normotensive group ($p = 0.043$, 0.010, 0.010, and 0.010, respectively) and also these parameters were significantly higher in the EOP group than in the LOP group ($p = 0.010$, for all variables). There were no significant differences in the mean values of the serum creatinine, hematocrit, and BUN among all groups ($p > 0.05$, Table 2). The serum fibronectin concentrations were significantly higher in the EOP group (30.22 ± 8.29 ng/ml) than in the LOP group (20.33 ± 5.11

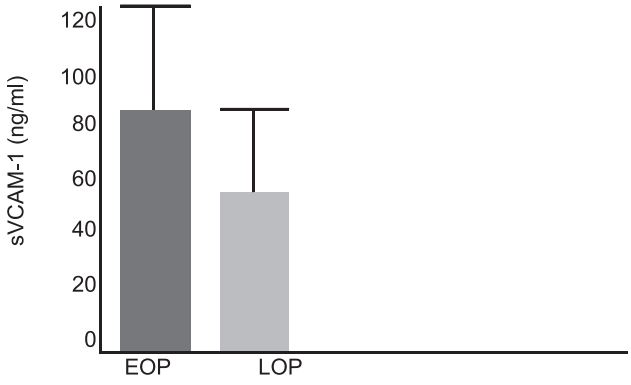

Figure 2. — Serum concentrations of sVCAM-1 in EOP and LOP (p = 0.01).

ng/ml) (p = 0.01, Figure 1). The serum sVCAM-1 concentrations (87.27 ± 38.11 ng/ml) were significantly higher in the early- onset preeclamptic group than in the LOP group (50.59 ± 33.22 ng/ml) (p = 0.01, Figure 2).

In the preeclamptic patients, the authors determined a positive correlation between plasma fibronectin values and the systolic and diastolic blood pressure measurements (r = 0.43 and 0.44, respectively) and they also found a positive correlation between sVCAM-1 and the systolic and diastolic blood pressure measurements (r = 0.54 and 0.64, respectively, Table 3). It was found that neonatal mortality and morbidity were significantly higher in the EOP group than in the LOP group (p = 0.002, Table 4).

Table 3. — *Plasma fibronectin and sVCAM-1 blood pressure correlation in study group.*

	Sperman's rho	Fibronectin	sVCAM-1
Systolic blood pressure	r	0.43	0.54
	p	0.0001	0.0001
Diastolik blood pressure	r	0.44	0.64
	p	0.0001	0.0001

r: correlation coefficient

Table 4. — *The comparison of neonatal morbidity and mortality between the early-onset and late-onset preeclamptic groups.*

	Early-onset preeclampsia (n=43)	Late-onset preeclampsia (n=37)	Total
Neonatal mortality	5	0	5
Neonatal morbidity	18	4	22
Healthy infant	20	33	53
Total	43	37	80

Pearson Chi-Square Test (p = 0.002)

Discussion

Despite the still unexplained pathogenesis, preeclampsia is thought to be the result of generalized endothelial dysfunction [12]. Increased levels of cell adhesion molecules, which are the excessive production of underperfused placenta, are believed to be indicators of endothelial dysfunction in preeclampsia [13]. In the present study, serum concentrations of sVCAM-1 were significantly higher in the preeclamptic group, especially EOP group. Lyall *et al.* are the first to show that sVCAM–1 is elevated in the serum of preeclampsia patients [7]. There are numerous studies that show an increase of adhesion molecules in preeclamptic pregnants [14-17]. Few studies have investigated the relationship between the markers of endothelial dysfunction and the severity of preeclampsia or pregnancy outcomes. Djurovic *et al.* published that maternal concentrations of sVCAM-1 were significantly elevated in both mild and severe preeclampsia and also in preeclampsia with SGA infants [18]. In a study by Shin-Young Kim *et al.*, it was revealed that sVCAM-1 was statically meaningful in estimating preeclampsia progression [19].

Measurement of plasma fibronectin level, which is the indicator of endothelium cell damage in the recognition of preeclampsia, showed an increase in the fibronectin levels in the preeclampsia cases [20-23]. In the present study, serum concentrations of fibronectin were significantly higher in the LOP group than in the normotensive group and they were also significantly higher in the EOP group than in the LOP group. In a study conducted by Power *et al.*, the effect of fibronectin level on adverse pregnancy outcome was examined in the preeclampsia patients and it was seen that elevated fibronectin was prevalent among the women with preeclampsia and the women were identified to be at increased risk for preterm delivery and SGA [24].

Fibronectin and sVCAM-1 levels were observed to be significantly high in direct proportion to the severity and the onset of the diseases. The present results demonstrated that the evidence of endothelial dysfunction in the women with EOP was associated with increased risk of adverse pregnancy outcomes.

Several investigators have proposed that EOP and LOP may have different pathophysiology and that these two phenotypes should be studied individually [3, 25, 26]. EOP is associated with greater perinatal and maternal mortality and morbidity than late-onset disease [27-29]. The present authors found that neonatal mortality and morbidity were significantly higher in the EOP than in the LOP group (p = 0.002). As expected, there was a positive correlation between plasma fibronectin and sVCAM-1 and the blood pressures in this study, which may be attributed to the hypertension-inducing effects of adhesion molecules.

Govender *et al.* investigated the role of angiogenic, antiangiogenic and vasoactive factors in the black South African women with EOP and LOP. They suggested that the excess of serum sFlt-1 and reduced VEGF and PlGF levels favored an anti-angiogenic state and endothelial dys-

function leading to preeclampsia and that the aetiology and pathogenesis of EOP and LOP differ [30]. Groten *et al.* investigated the expression of VE-cadherin and vascular endothelial growth factor receptor-2 (VEGFR2) in preeclampsia. The findings of their study lead the present authors to conclude that the etiology and pathogenesis of EOP and LOP are to some extent different [31].

The present study is the first to demonstrate that the concentrations of fibronectin and sVCAM-1 are higher in the EOP group than in the LOP group. These different values between EOP and LOP seem to support the opinion that physiopathology of both group diseases differs from one another according the prognoses and results.

References

[1] Sibai BM.: "Diagnosis and management of gestational hypertension and preeclampsia". *Obstet. Gynecol.*, 2003, *102*, 181.

[2] Raymond D., Peterson E.: "A critical review of early-onset and late-onset preeclampsia". *Obstet. Gynecol. Surv.*, 2011, *66*, 497.

[3] Von Dadelszen P., Magee L.A., Roberts J.M.: "Subclassification of preeclampsia". *Hypertens. Pregnancy,* 2003, *22*, 143.

[4] Roberts J.M., Gammill H.: "Preeclampsia and cardiovascular disease in later life". *Lancet*, 2005, *366*, 961.

[5] Conrad K.P., Benyo D.F.: "Placental cytokines and the pathogenesis of preeclampsia". *Am. J. Reprod. Immunol.*, 1997, *37*, 240.

[6] Thornhill M.H., Haskard D.O.: "IL-4, regulates endothelial cell activation by IL-1, tumor necrosis factor or IFN-gamma". *J. Immunol.*, 1990, *145*, 865.

[7] Lyall F., Greer I.A., Boswell F., Macara L.M., Walker J.J., Kingdom J.C.: "The cell molecule, VCAM 1 is selectively elevated in serum preeclampsia: does it indicate the mechanism of leucoyt activation?" *Br. J. Obstet. Gynaecol.*, 1994, *101*, 485.

[8] Redman C.W., Sargent I.L.: "Placental stress and pre-eclampsia: a revised viev". *Placenta*, 2009, *30*, S38.

[9] Frenette P.S., Wagner D.D.: "Adhesion molecules–part I". *N. Engl. J. Med.*, 1996, *334*, 1527.

[10] Holtfreter J.: "Significance of the cell membrane in embrionic processes". *Ann. N.Y. Acad. Sci.*, 1948, *49*, 709.

[11] Elices M.J., Osborn L., Takada Y., Crouse C., Luhowskyj S., Chii Rosso G., Lobb R.R.: "VCAM-1 on activated endhotelium interacts leucocyt integrin VLA-4 at a site distinct from VLA-4 /fibronectin binding site". *Cell*, 1990, *60*, 577.

[12] Lyall F., Greer I.A.: "Preeclampsia a multifacted vasculer disorder of pregnancy". *J. Hypertens.*, 1994, *12*, 1339.

[13] Harris L.K., Jones C.J.P., Aplin J.D.: "Adhesion molecules in human trophoblast - a review. II. extravillous trophoblast.". *Placenta*, 2009, *30*, 299. doi: 10.1016/j.placenta.2008.12.003. Epub 2009 Jan 7.

[14] Zhang Y., Gu Y., Lewis D.F., Wang Y.: "Reduced cellular glutathione reductase activity and increased adhesion molecules expression in endhotelial cells cultured with maternal plasma from woman with preeclampsia". *Soc. Gynecol. Investig.*, 2006, *13*, 412. Epub 2006 Jul 31.

[15] Vadachkoria S., Woelk G.B., Mahomed K., Qiu C., Muy-Rivera M., Malinow M.R., Williams M.A.: "Elevated soluble vascular cell adhesion molecule-1, elevated Homocyst(e)inemia, and hypertriglyceridemia in relation to preeclampsia risk". *Am. J. Hypert.*, 2006, *155*, 235.

[16] WilczyDski J.R., Głowacka E., Nowak M., Szpakowski A.: "Serum concentration of soluble vasculer celluler adhesion molece 1and expretion of ıts receptor VLA 4 on the surface of peripheral blood and decidual lymphocytes of preeclamptic woman". *Ginekol. Pol.*, 2000, *74*, 1335.

[17] Heyl W., Heintz B., Reister F., Harwig S., Witte K., Lemmer B., Rath W.l.: "Increased soluble VCAM 1serum levels in preeclampsia are not correleted to urinary excretion or circadian bloood pressure rthym". *J. Perinat. Med.*, 2005, *33*, 144.

[18] Djurovick S., Scjhetlein R., Wisloff F., Haugen G., Berg K.: "Increased levels of intercelluler adhesion molecules in preeclampsia". *Br. J. Obstet. Gynecol.*, 1997, *104*, 466.

[19] Kim S.Y., Ryu H.M., Yang J.H., Kim M.Y., Ahn H.K., Lim H.J., et al.: "Maternal serum levels of VCAM 1, ICAM 1 and E Selectin in preeclampsia". *J. Korean Med. Sci.*, 2004, *19*, 688.

[20] Saleh A.A., Bottoms S.F., Norman G., Farag A.: "Mammen EF. Hemostasis in hypertensive disorders of pregnancy". *Obstet. Gynecol.*, 1988, *71*, 719.

[21] Stubbs T.M., Lazarchick J., Horger E.O.: "Plazma fibronektin levels in preeclampsia: A possible biochemical marker for vasculer endhotelial damage". *Am. J. Obstet. Gynecol.*, 1984, *150*, 885.

[22] Brubaker D.B., Ross M.G., Marinoff D.: "The function of elevated plasma fibronectin in preeclampsia". *Am. J. Obstet. Gynecol.*, 1992, *166*, 526.

[23] Saleh A.A., Bottoms S.F., Welch R.A., Ali A.M., Mariona F.G., Mammen E.F.: "Preeclampsia, delivery and the hemoststic system". *Am. J. Obstet. Gynecol.*, 1987, *157*, 331.

[24] Powers R.W., Catov J.M., Bodnar L.M., Gallaher M.J., Lain K.Y., Robberts J.M.: "Evidence of endothelial dysfunction in preeclampsia and risk of adverse pregnancy outcome". *Reprod. Sci.,* 2008, *15*, 374.

[25] Vatten L.J., Skjaerven R.: "Is preeclampsia more than disease?" *BJOG*, 2004, *111*, 298.

[26] Valensise H., Vaspollo B., Gagliardi G., Novelli G.P.: "Early and late preeclampsia: two different maternal hemodynamic states in latent phase of the disease". *Hypertension*, 2008, *42*, 873.

[27] MacKay A.P., Berg C.J., Atrash H.K.: "Pregnancy-related mortality from preeclampsia". *Obset. Gynecol.*, 2001, *97*, 533.

[28] Odegard R.A., Vatten L.J., Nilsen S.T., Salvesen K.A., Austgulen R.: "Preeclampsia and fetal growth". *Obstet. Gynecol.*, 2000, *96*, 950.

[29] Mbah A.K., Alio A.P., Marty P.J., Bruder K., Whiteman V.E., Salihu H.M.: "Pre-eclampsia in the first pregnancy and subsequent risk of stillbirth in black and gravidas". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2010, *149*, 165.

[30] Govender L., Mackrai I., Gathiram P., Moodley J.: "The role of angiogenic, antiangiogenic and vasoactive factors in preeclamptic African women: early-versus late-onset preeclampsia". *Cardiovasc. J. Afr.*, 2012, *23*, 153.

[31] Groten T., Gebhard N., Kreienberg R., Schleussner E., Reister F., Huppertz B.: "Differential expression of VE- cadherin and VEGFR2 in placental syncytiotrophoblast during preeclampsia – Newperspectives to explain the pathopysiology". *Placenta*, 2010, *31*, 339.

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