

Thrombophilia Markers in Preeclampsia

Preeklampside Trombofili Belirteçleri

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ABSTRACT Objective: To evaluate the role of coagulation parameters and genetic markers of thrombophilia such as Factor V Leiden (FVL) and the prothrombin gene (PG) mutations in pregnancy associated hypertension. **Material and Methods:** A total of 116 pregnant women were included in this study: 63 were pregnancy induced hypertension (PIH), and a further 53 were healthy pregnant women as control group. **Results:** Blood urea nitrogen (BUN), serum creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) values were significantly elevated in women with PIH compared to control group. Additionally hemoglobin and platelet counts were significantly decreased in women with PIH. Although plasma protein C (PC), protein S (PS), and activated protein C resistance (APCR) were similar, we detected statistically decreased levels of plasma antithrombin III (AT III) and increased levels of D-Dimer in preeclamptic groups. Lastly prevalence of FVL and PG mutations in PIH was not higher than control group. **Conclusion:** In the present report, the frequency of common inherited thrombophilias in preeclampsia such as FVL and PG mutations were studied. Also the variations between the measurements of PC, PS, APCR, ATIII, and D-Dimer were investigated in preeclampsia.

Key Words: Preeclampsia; thrombophilia; genetic markers

ÖZET Amaç: Gebeliğe bağlı hipertansiyonda koagülasyon parametreleri ile Faktör V Leiden (FVL) ve protrombin gen (PG) mutasyonları gibi trombofili genetik belirteçlerin değerlendirilmesi. **Ge-reç ve Yöntemler:** Bu çalışmaya 63'ü gebeliğin indüklediği hipertansiyonu (GİH) olan ve 53 sağ-lıklı gebenin kontrol grubu olduğu toplam 116 gebe dahil edildi. **Bulgular:** Kan üre nitrojen (KÜN), serum kreatinin (KRE), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve laktat dehidrojenaz (LDH) değerleri kontrol grubu ile karşılaştırıldığında GİH olan hastalarda anlamlı olarak artmış bulunmuştur. Ek olarak, hemoglobin ve trombosit sayısı GİH olan hasta-larda anlamlı olarak azalmıştır. Plazma protein C (PC), protein S (PS) ve aktive protein C diren-ci (APCD) benzer olmasına karşın preeklamptik grupta azalmış plazma antitrombin III seviyelerini ve artmış D-Dimer seviyelerini istatistiksel olarak anlamlı tespit ettik. Son olarak GİH'de FVL ve PG mutasyonlarının prevalansı kontrol grubundan yüksek değildir. **Sonuç:** Bu çalışmada, preeklampside kalıtsal trombofililerden FVL ve PG mutasyonlarının sıklığı araştırılmıştır. Ayrıca PC, PS, APCD, ATIII ve D-Dimer seviyelerindeki preeklamptik gruptaki farklılıklar gözden geçirilmiştir.

Anahtar Kelimeler: Preeklampsi; trombofili; genetik belirteçler

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Preeclampsia is a systemic disorder characterized by hypertension, edema and proteinuria in which a diffuse vascular damage with deposition of fibrin is well-recognized pathological feature. It can ha-

ve a major impact on both perinatal and maternal morbidity and prevalence are 5-7% of all pregnancies.^{1,2}

Recently many of the authors claim that abnormalities of natural coagulation inhibitor levels and genetic parameters create a great tendency towards preeclampsia which is related with adverse pregnancy outcome such as thrombosis.³

PC/PS anticoagulant pathway is a central component and also a regulatory network in order to limit clot formation. Factors (F) Va and VIIIa inactivation is the major function of this pathway.⁴ AT III is a major physiological inhibitor of coagulation.⁵ PC is activated to activated protein C (APC) by thrombin in the presence of thrombomodulin. APC with PS and FV as cofactors inactivate FVa and FVIIIa.⁶

FVL has been associated with a number of early and late obstetrical complications such as preeclampsia and haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome.^{7,8} There is also an increase in the level of circulating fibrin degradation products (FDP), produced as a result of secondary to fibrinolysis in preeclampsia and HELLP syndrome.⁹

The most commonly identified inherited thrombophilias, related to preeclampsia; consist of FVL and the prothrombin gene mutation (PGM).¹⁰ Some abnormalities might require having anticoagulant therapy or antiplatelet therapy when detection of abnormal thrombophilia markers was made.

The aim of this study was, to detect clinical evaluation of coagulation inhibitors and genetic parameters in preeclamptic and normotensive pregnant women, to determine their important role in pathogenesis of preeclampsia.

MATERIAL AND METHODS

A total of 116 pregnant women between 26th and 40th weeks of gestation admitted to our department were investigated. 53 pregnant women with uncomplicated healthy pregnancies constituted our

control group and 63 women with undefined type of preeclampsia constituted our study group. Women who reported histories of preeclampsia were not eligible to be controls. There were no significant differences between study and control group for age, parity, in utero exitus and gestational age. Patients with multiple pregnancies, with chronic renal and vascular disease or previous thromboembolic complications were excluded and women who is taking anticoagulant therapy or having preeclampsia superimposed on chronic hypertension were not included in the study. None of the patients or controls was in labor at the time of sampling. All patients delivered in the Obstetrics Department of Erciyes University (EU) Faculty of Medicine and were followed until discharged from hospital.

After all patients were informed about trial and got their contents; 12 mL of blood was drawn from the antecubital vein into plastic tubes at admission and diluted with 1 mL 3.8% sodium citrate. To avoid interassay and intraassay variation, samples of AT III, PC and total PS activities, APC sensitivity ratio and D-Dimer measurements were assayed at the same time by the same technician and again pregnant women were tested for FVL and prothrombin mutation at the same time by the same technician. Our genetic department was able to concluded FVL and prothrombin mutation tests 53 pregnant women of 63 pregnant women in preeclamptic group and 45 of 53 pregnant women in control group because of inadequate DNA derivation.

PC, PS, APC, AT III and D-Dimer were measured by using ERCEP bioMérieux (Durham/North Carolina/USA) kit using coagulometer analyzer (MDA II® Platelin® LS Durham/North Carolina/USA) at the clinical oncology laboratory of our institution. We also tested APC, PS with a clotting method, AT III, PC with a chromogenic method, and D-Dimer with an immunological method. To detect FVL and prothrombin mutations, we used allele-specific PCR amplification (ASA-PCR) by using PCR analyzer (Bio-rad i cycler Hercules/California/USA). DNA isolation kit (EZNA®,

Ankara/Turkey) was used for DNA isolation in the Department of Genetic Diseases.

Diagnosis of preeclampsia was done according to the criteria agreed by the National High Blood Pressure Education Program Working Group of National Institutes of Health (NIH) in 2000.¹¹ Preeclampsia was defined as blood pressure (BP) of at least 140/90 mmHg after 20 weeks gestation on at least two occasions 6 hours apart when the absence of gestational trophoblastic disease or multiple pregnancies was confirmed by ultrasonographic examination, with proteinuria more than 0.3 g per 24 hours and edema < 1+ after bed rest. Blood was measured with a calibrated aneroid manometer in the supine position after five minutes rest. Absolute diastolic BP of ≥ 110 mmHg and proteinuria ($\geq 2+$ [100 mg/dL]) on a catheterized specimen was diagnostic for severe preeclampsia at admission.

Whereas patients with preeclampsia had grand-mal seizure were defined as eclampsia. When the absence of gestational trophoblastic disease or multiple pregnancies was confirmed by ultrasonographic examination, patient with >140/90 mmHg tension before pregnancy or 20th weeks of

gestation were classified as chronic hypertension. Gestational age was estimated from the first day of last menstrual period and confirmed by ultrasonographic measurements.

All of continuous variables were subjected to normality testing using the Kolmogorov-Smirnov method and data were expressed as mean \pm SD and median (min-max). Continuous variables were analyzed with non-parametric methods. Differences between control and preeclamptic groups were evaluated with Mann Whitney U test and chi-square was used for comparing categorical values. P values by Fischer exact test were reported for 2 x 2 tables when the assumptions for the chi-square analysis were not met. Data were stored and analyzed with the Statistical Package for Social Sciences (SPSS), release 13.0 (Chicago IL) for Windows. Statistical significance was defined as $p < 0.05$. This study was approved by the Ethics Committee for Human research at EU, Turkey.

RESULTS

As illustrated in table one, BUN, CRE, AST, ALT, LDH values, hemoglobine and platelet counts were significantly different between the groups. BUN,

TABLE 1: Mean \pm SD and Median (minimum-maximum) of blood urea nitrogene, serum creatinin, AST, ALT, LDH, hemoglobin and platelet counts in the groups.

	Control (n= 53)	Preeclampsia (n= 63)	p
Hemoglobin	11.84 \pm 1.41	11.00 \pm 2.06	0.009
	12.10 (8.90-14.10)	11.00 (6.00-15.00)	
Platelet	224.83 \pm 57.55	190.56 \pm 81.73	0.005
	218.00 (87.00-371.00)	194.00 (27.00-463.00)	
Blood urea nitrogen	7.28 \pm 4.18	12.29 \pm 6.13	0.001
	6.00 (3.00-27.00)	11.00 (4.00-44.00)	
Serum creatine	0.58 \pm 0.14	0.79 \pm 0.22	0.001
	0.50 (0.20-0.90)	0.70 (0.50-1.60)	
AST	23.42 \pm 23.40	73.42 \pm 146.54	0.001
	19.00 (10.00-179.00)	32.00 (12.00-1069.00)	
ALT	20.96 \pm 21.83	65.97 \pm 13664	0.004
	16.00 (7.00-124.00)	22.00 (5.00-828.00)	
LDH	220.17 \pm 184.02	532.97 \pm 504.07	0.001
	172.00 (105.00-1379.00)	372.00 (101.00-3269.00)	

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase.

CRE, AST, ALT and LDH levels were significantly elevated in women with preeclampsia, severe preeclampsia or eclampsia compared to healthy controls. Hemoglobin and platelet counts were significantly decreased in women with preeclampsia, severe preeclampsia or eclampsia compared to healthy controls (Table 1).

As shown on Table 2, mean \pm SD values for PC, PS and APCR were similar in the pregnancies both preeclampsia and control. There was no statistical significance between control and study groups for preeclampsia. There was statistical difference for the plasma AT-III and D-Dimer levels among these three different examinations ($p < 0.05$). We were also detected decreased levels of plasma AT III and increased levels of D-Dimer in preeclamptic groups (Table 2).

Thirty nine of 63 (61.9%) patients in the preeclamptic group, 29 of 53 (54.7%) patients in the healthy pregnancies ($p = 0.553$) had APCR ratio under the suggested value of 2.1. As a result the

frequency of APCR was not significantly different between these examinations.

Table 3 shows the correlation between preeclampsia and genetic mutations. Although the prevalence of each groups of preeclampsia was higher than control groups, there was no statistical significance between control and study groups for early preeclampsia. Additionally we were not able to show any prothrombin mutation in control group, there was no statistical significance between control and study groups for prothrombin mutation ($p = 0.247$).

DISCUSSION

Preeclampsia is associated with endothelial injury and haemostatic abnormalities which may in turn predispose the subjects to thrombosis. However, the extent of the coagulation problems induced by hypertensive disorders of pregnancy is not clear yet. Therefore a lot of evaluations were found for this disorder.

TABLE 2: Mean \pm SD and Median (minimum-maximum) protein C, protein S, antithrombin III activities, activated protein C resistance, and D-Dimer values in pregnancies.

	Control (n= 53)	Preeclampsia (n= 63)	p
Protein C	120.56 \pm 23.76 117.21 (191.77-74.10)	121.33 \pm 28.19 119.00 (72.30-203.10)	0.958
Protein S	79.18 \pm 37.43 79.00 (12.21-210.00)	82.08 \pm 44.76 78.00 (14.69-232.00)	0.923
Activated protein C resistance	2.12 \pm 0.63 2.03 (0.86-3.53)	1.99 \pm 0.60 1.95 (0.86-3.23)	0.294
Antithrombin III	109.00 \pm 16.51 110.16 (71.45-142.10)	95.51 \pm 23.17 96.10 (10.02-141.00)	0.001
D-Dimer	1.28 \pm 0.82 1.03 (0.36-4.24)	4.06 \pm 6.03 2.70 (0.70-40.00)	0.001

n= number of patients.

TABLE 3: Genetic evaluations in preeclamptic and control groups.

Total genetic evaluation	Preeclamptic group	Control group	p
FVL positive	9 (17%)	5 (11.1%)	> 0.05
FVL negative	44(83%)	40 (88.9%)	> 0.05
PGM positive	3 (5.7%)	0 (0%)	> 0.05
PGM negative	50 (94.3%)	45 (100%)	> 0.05

FVL: Factor V Leiden, PGM: Prothrombin Gene Mutations.

As data presented in the table one; serum AST, ALT, LDH, BUN and CRE levels were elevated, platelet and hemoglobin accounts were decreased in women with preeclampsia compared to healthy controls. These data were in agreement with the declarations of National Institutes of Health in which major criteria of preeclampsia were defined, include all above data except for hemoglobin and BUN levels.¹¹

In studies, PC and AT III results in pregnancy associated hypertensive disorders were reported differently including a significant increase in PC levels with preeclampsia, a significant decrease or no difference in PC and AT III levels in preeclampsia and severe preeclampsia as compared to normal pregnancies.¹²⁻¹⁸ In our study, we observed that the plasma PC level was higher in women with preeclampsia than in controls, but the average levels were in normal limits of references. And we did not find statistically significance between control and preeclamptic groups for PC levels. Additionally the plasma level of AT III was lower in women with preeclampsia than in controls and this value reflected statistically significance between the groups.

The decreased activity in PC and AT III was described by the consumption of these coagulation inhibitors due to activation of coagulation and the resultant disseminated intravascular coagulation (DIC).^{14,15,19} Besides, AT III production may decrease as a result of severe preeclampsia.²⁰ We observed decreased AT III activity in women with pregnancy associated hypertensive disorders, this was statistically significant. On the other side PC activity increased in women with pregnancy associated hypertensive disorders despite the expected decrease in coagulation inhibitors. Also compensatory increase in PC activity might have balanced the decreased PC activity in our patients, could be the possibly explaining of this mechanism.

Also there was a correlation between PC activity and PS in both preeclamptic patient groups. This was supported by the findings of the different

studies, but was conflicting with other studies.^{13,21-23} The compensatory increase in the coagulation inhibitors with severe preeclampsia which may partly balance the state of hypercoagulation, might explain the high levels of PC and total PS in severe preeclampsia. In our study, we found that the plasma levels of PS were elevated in preeclamptic group compared to controls likewise PC but there was not statistically significance between groups.

As previously reported, APCR was related with an important role of the pathogenesis of preeclampsia.^{24,25} However we did not demonstrate a direct relation between APCR and preeclampsia like other previous investigators.^{26,27} Ethnic differences among the study groups as well as the different inclusion and exclusion criteria employed by different investigators explain why the result of APCR were similar between women with pregnancy associated hypertension and healthy pregnant women. The divergent results of our study from the literature might be a result of different reference values of APCR.²¹

The coagulation system activity in pregnancy is increased with thrombin and fibrin formation and degradation which occurring continuously the fibrinolytic process in preeclampsia is more prominent.^{28,29} Immune complex deposition in endothelial tissue with subsequent damage and further triggering of fibrin formation may be the cause of increased coagulability in preeclampsia.³⁰ When fibrin degradation is occurred, D-Dimer is derived and may show the rate of fibrin formation. And the hypercoagulability state in preeclampsia might elevate D-Dimer levels. Therefore D-Dimer levels might increase in PIH similarly disseminated intravascular coagulation. We detected that D-Dimer levels in preeclamptic groups were more marked than controls and this increased levels of D-Dimer were statistically significant between groups.

Inherited thrombophilia is a heterogeneous group of conditions that have been implicated in a variety of pregnancy complications. Evidence is

mounting that implicates these inherited disorders in a range of pregnancy outcomes, including recurrent miscarriage, late fetal loss, preeclampsia, abruptio placenta, and intrauterine growth restriction. The most commonly identified inherited thrombophilias consist of FVL and PGM.³⁰ In contrast, there are also several studies, which reported no association of FVL with preeclampsia.^{31,32} In our series; we found FVL mutation in 9 of 53 (17%) women and when we compared to the control group there was no statistically significant difference. Similarly, the majority of studies found no significant association between

preeclampsia and PGM.^{33,34} In our series, %5.7 of women with preeclampsia was affected and this mutation should not involve in the relation of preeclampsia.

In conclusion, the frequencies of common inherited thrombophilias among women with preeclampsia are determined in this study. Although further studies might need to confirm, our results about FVL and PGM may not support the view that these mutation might contribute to the pathogenesis of preeclampsia. Also, it seems that APCR may not be adequate to make definite comments in preeclampsia.

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