The relationship between thrombophilic mutations and preeclampsia: a prospective case-control study

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BACKGROUND: Preeclampsia and its association with thrombophilia remain controversial, due to inconsistent results in different studies, which include different ethnic groups, selection criteria, and patient numbers. The aim of this study was to determine the relationship between thrombophilia and preeclamptic patients in our region.

METHODS: In a prospective case-control study, we compared 100 consecutive women with preeclampsia and eclampsia (group 1) with 100 normal pregnant women (group 2). All women were tested two months after delivery for mutations of factor V Leiden, methylenetetrahydrofolate reductase (MTHFR), and prothrombin gene mutation as well as for deficiencies of protein C, protein S, and antithrombin III.

RESULTS: A thrombophilic mutation was found in 42 (42%) and 28 (28%) women in group 1 and group 2, respectively (P=0.27, OR 1.5, 95%CI 1.0-2.2). The incidence of Factor V Leiden mutation (heterozygous), prothrombin mutation (heterozygous), prothrombin mutation (homozygous), MTHFR mutation (homozygous) was not statistically significant in group 1 compared with group 2 (P>0.05). Also, deficiencies of protein S, protein C, and antithrombin III were not statistically significant in group 1 compared with group 2 (P >0.05).

CONCLUSION: There was no difference in thrombophilic mutations between preeclamptic patients and normal pregnant women in our region. Therefore, we suggest that preeclamptic patients should not be tested for thrombophilia.

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Preeclampsia is an important cause of maternal and fetal morbidity and mortality. Although the etiology of preeclampsia remains unknown, it has been suggested that preeclampsia is associated with intervillous and spiral artery thrombosis, vascular endothelium damage and abnormalities of coagulation, leading to inadequate maternal, fetal and placental circulation.[1] The term thrombophilia describes disorders of the haemostatic mechanisms that are likely to predispose to thrombosis.[2] Preeclampsia and its association with thrombophilia remain controversial. Several investigators have reported an association between thrombophilia and adverse pregnancy outcomes caused by uteroplacental thrombosis, such as severe intrauterine growth restriction and placental abruption. However, other groups have failed to confirm this association.[3,4,5] These inconsistent results may reflect the varying ethnic groups, selection criteria, and the number of cases included in different studies.[6,7] The primary objective of our study was to test whether an association exists between preeclampsia, eclampsia and thrombophilia in a population of women with preeclampsia and

eclampsia in the Southeast of Turkey. Therefore, we studied mutations for factor V Leiden, prothrombin, methylenetetrahydrofolate reductase (MTHFR) and deficiencies of the natural anticoagulant proteins C, S, and antithrombin.

Methods

This study was conducted between September 2004 and April 2005 in the southeast of Turkey. The people of this region are of Kurdish, Arabic and Turkish origin. We studied 100 consecutive women with a singleton pregnancy complicated by severe preeclampsia and eclampsia (group 1) and 100 consecutive healthy normotensive pregnant women (group 2). The healthy normotensive pregnancies were diagnosed on the basis of clinical, biochemical, and ultrasound findings and none of the healthy pregnant had pre-existing hypertensive disorders or any renal, hepatic, or hematological diseases or a thromboembolic event. All patients referred to our clinic were included in study. Preeclampsia, eclampsia and HELLP syndrome (hemolysis, elevated liver enzymes and low platelets) were determined using the criteria of the American College of Obstetricians.[8] The study and control women were enrolled during their stay in the hospital after delivery.

Two months after delivery, blood samples were taken from each woman, and samples for analysis of natural anticoagulant proteins C, S, and antithrombin III were collected into appropriate tubes and centrifuged for 5 minutes at 3000Xg to separate serum and plasma. Samples were collected into EDTA containing tubes for factor V Leiden, MTHFR and prothrombin gene mutation analysis. All blood samples were stored at -20˚C until analysis. Genomic DNA was prepared from peripheral blood samples using a High Pure PCR Template Preparation DNA kit (Roche Diagnostics GmbH, Penzberg, Germany) following manufacturer's protocol.

The presence of methylenetetrahydrofolate reductase C→T 677 mutation detection was evaluated using an MTHFR detection kit (Roche Diagnostics Corporation with real-time PCR using a LightCycler Instrument, Roche Diagnostics Corporation, Germany). The presence of Factor V Leiden mutation was evaluated by a Factor V Leiden kit (Roche Diagnostics Corporation with real-time PCR using a LightCycler Instrument, Roche Diagnostics Corporation, Germany). The presence of a prothrombin mutation was evaluated using a Factor II (prothrombin) G20210A kit (Roche Diagnostics Corporation with Real-time PCR using a LightCycler Instrument, Roche Diagnostics Corporation, Germany). Protein C and protein S levels were measured by an automated functional clotting assay for the quantitative determination of protein C and protein S in human plasma (HemosIL test for protein C [reference range, 71.8%-146.2%] and the HemosIL test for protein S [reference range 64.4-128.8%], Instrumentation Laboratory, Italy). The antithrombin III level was measured by a chromogenic assay (HemosIL test for antithrombin III [reference range 84.6%-120.2%], Instrumentation Laboratory, Italy).

The results of the two groups were compared by the two-tailed Student *t* tests, and the Pearson χ^2 test (or the Fisher exact test, if the expected count was less than 5). Odd ratios (OR) and 95% confidence intervals (95%CI) were calculated. Numerical samples were analyzed by Student *t* tests, but categorical samples were analyzed by the Pearson χ^2 (or Fisher exact test). Statistical analyses were performed with the SPSS statistical package for Windows, version 10.0.

Results

In group 1, the gestational week at delivery and birth weight were significantly lower ($P<0.001$) than in group 2 (Table 1). Group 1 had a blood pressure (BP) >140/90 mm Hg and proteinuria. Preeclampsia was characterized as severe with of a BP > 160/110 mm Hg in 23 women, proteinuria in excess of 5 g/24 hours in 8 women, a platelet count <100 000/mm^3 in 7, HELLP syndrome in 3, elevated liver enzymes in 6 and eclampsia in 19.

The incidence of Factor V Leiden mutation (heterozygous), prothrombin mutation (heterozygous), prothrombin mutation (homozygous), and MTHFR mutation (homozygous) was not statistically significant in the study group compared with the control group ($P>0.05$) (Table 2). In addition, deficiencies of protein S, protein C, and antithrombin III were not statistically significant in the study group compared with the control group ($P>0.05$). Overall, 26 study women (26%) had one of the three thrombophilic mutations compared with 16 control women (16%), ($P=0.118$, OR 1.6, 95%CI 0.9-2.8). The combined prevalence of all inherited and acquired thrombophilia in the study women was 42% compared with 28% in the control group (OR 1.5, 95%CI 1.0-2.2) (Table 2). In addition, 16 women (16%) had other types of inherited or acquired thrombophilia compared with 12 control woman (12%), ($P=0.271$, OR 1.3, 95%CI 0.6-2.6). Two women in group 1

Table 1. Clinical characteristics of severe preeclampsia and normal pregnancy

Characteristics	Preeclampsia and eclampsia (Group 1)	Normal Pregnancy (Group 2)	P
Age	30.28±6.40	29.70±5.59	0.536
Gravida	5.00±3.54	4.90±3.40	0.892
Parity	3.37±3.34	3.02±3.13	0.885
Mean systolic arterial pressure (mm Hg)	158.30±5.3	113.70±3.9	0.000
Mean diastolic arterial pressure (mm Hg)	108.40±9.1	78.40±6.5	0.0001
Gestational weeks at delivery	34.15±4.09	38.06±1.29	0.0001
Apgar at 1 minute	4.65±2.65	6.01±1.94	0.0001
Apgar at 5 minutes	6.42±3.05	8.13±1.46	0.0001
Birth weight (g)	2228.50±88.86	3245.54±398.68	0.0001

Data are mean±SD [AUTHOR: Please verify that this is correct]

Table 2. Prevalence of inherited and acquired thrombophilia in normal pregnancy and women with preeclampsia and eclampsia.

Thrombophilia	Preeclampsia and eclampsia (n=100)	Normal Pregnancy (n=100)	Odds ratio (95%CI)	P
Factor V Leiden mutation (heterozygous)	6	4	1.5 (0.4-5.1)	0.748
Prothrombin mutation (heterozygous)	4	1	4.0 (0.4-35.1)	0.369
Prothrombin mutation (homozygous)	0	1	1.0 (0.9-1.0)	1.0
MTHFR (homozygous)	16	12	1.3 (0.6-2.6)	0.271
All genetic mutations	26	16	1.6 (0.9-2.8)	0.118
Protein S	14	12	1.1(0.5-2.3)	0.834
Protein C	1	0	1.0 (0.9-1.0)	1.0
Antithrombin III	1	0	1.0 (0.9-1.0)	1.0
All thrombophilia	42	28	1.5(1.0-2.2)	0.27

(including one patient with factor V Leiden mutation and protein S deficiency, and one patient with prothrombin mutation and protein S deficiency) had combined thrombophilia versus none in the group 2 (*P* =0.497, OR 0.9; 95%CI 0.9-1.0). Two women in group 1 (including one patient with factor V Leiden mutation and one with protein S deficiency) had stillbirth versus none in group 2 (*P* =0.497, OR 0.9, 95%CI 0.9-1.0). Three placental abruptions were found in group 1 (including one patient with one prothrombin mutation, one antithrombin III deficiency and one protein C deficiency) versus none in group 2 (*P* =0.246, OR 0.9; 95%CI 0.9-1.0). Three women in group 1 had intrauterine growth restriction (IUGR) and protein S deficiency versus none in group 2 (*P*=0.029, OR 0.9, 95% CI 0.8-0.9).

Discussion

Preeclampsia is a multisystem disorder involving vasoconstriction and hypertension in the mother and decreased blood flow. It occurs in 5% to 15% of pregnancies and is one of the major causes of maternal and fetal morbidity and mortality.[9] There are inconsistent reports on whether there is an association between preeclampsia and maternal or fetal thrombophilia. The relationship of Factor V Leiden to other disorders of pregnancy remains controversial. Kupferminc et al. reported a prevalence of 67% of

some form of thrombophilia in patients with severe preeclampsia versus 20% in controls and an odds ratio of 4.6 for the Factor V Leiden mutation.[10] Lindqvist et al. found no difference in prevalence for this mutation in preeclampsia or IUGR patients.[11] This finding is supported by the findings of Livingston et al who found no association of maternal or fetal genetic polymorphisms (Factor V Leiden, Factor II, MTHFR) and severe preeclampsia.[12]

A point mutation at nucleotide 1691 in exon 10 of the Factor V gene causes an amino acid substitution of glutamine for arginine at position 506 (R506Q). As a result, Factor Va will be resistant to proteolytic inactivation by activated protein C.[13] It has been suggested that the Factor V Leiden mutation, when enhanced by the physiological hypercoagulation in pregnancy, may contribute to increased thrombus formation in the placenta and thus may be a hereditary risk factor for preeclampsia.[2-5,17,18]

A possible association of the Factor V Leiden mutation with preeclampsia has been reported for different populations. However, the mutation is frequent in some ethnic groups but rare in other ethnic groups, where its relevance for the etiology of preeclampsia has not been established yet, based on the significant population-specific differences of the Factor V Leiden mutation.[14,15] Driul et al[16] had found higher prevalence of factor V Leiden mutation in women with preeclampsia compared with control subjects. However, a controversy exists with regard to the prevalence of Factor V Leiden mutation in preeclampsia[17-18]. In our patients the frequency of heterozygous carriers of the Factor V Leiden mutation was 6% in the women with preeclampsia compared with 4% in the patients with normal pregnancies. This difference was not statistically significant (*P*>0.05).

A congenital thrombophilic disorder recently described is the G20210A mutation in the Factor II (prothrombin) gene. The mutation leads to higher prothrombin production and to an elevated risk of thrombosis, but there are few previous reports on the prothrombin gene mutation in preeclampsia.[4-6] Livingston, et al[19] had found no association with severe preeclampsia and prothrombin gene mutation. On the contrary, Grandone et al[20] reported that the prothrombin gene mutation was significantly (*P*=0.003) more frequent in 70 preeclamptic subjects (14.3%) than in 216 controls (4.2%).

In our patients, the frequency of heterozygous prothrombin gene mutation was 4% in study group compared with 1% in the patients with normal pregnancies (odds ratio 4.0, %95CI 0.4-35.1). This difference was not statistically significant (*P*<0.369). Additionally, the frequency of the homozygous prothrombin gene mutation was 1% in study group compared with 0% in the study group (odds ratio 1.0, 95% CI 0.9-1.0).

The increased prevalence of homozygosity for the cytosine 677 thymine (C677T) mutation in MTHFR has been reported previously by several groups,[6-21] but has not been found in other studies.[22-23] In our patients the frequency of homozygosity in thr methylenetetrahydrofolate reductase (MTHFR) gene mutation was 16% in the study group compared with 12% in the patients with normal pregnancies (odds ratio 1.3, 95% CI 0.6-2.6). This difference was not statistically significant (*P*<0.271).

Sayin et al reported decreased protein S activity in women with hypertensive disorders of pregnancy compared with healthy controls, but no difference in protein C and antithrombin III activity.[24] In our patients deficiencies of protein S, protein C, and antithrombin III were not statistically significant in study group compared with the control group (P>0.05).

Our findings suggest that the combined prevalence of all inherited and acquired thrombophilia in the hypertensive group was 42% compared with 28% in the control group (OR 1.5, 95%CI 1.0-2.2). However, the incidence of Factor V Leiden mutation (heterozygous), prothrombin mutation (heterozygous), prothrombin mutation (homozygous), MTHFR mutation (homozygous) was not statistically significant in the hypertensive group compared with healthy pregnant women (*P* >0.05). In addition, deficiencies of protein S, protein C, and antithrombin III were not statistically significant in the study group compared with the control group (*P* >0.05). As a result, we do not recommend screening for thrombophilia in hypertensive disorders of pregnancy in our population.

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