The relationship between thrombophilic mutations and pre eclampsia: 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.

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. The term thrombophilia describes disorders of the haemostatic mechanisms that are likely to predispose to thrombosis. 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. These inconsistent results may reflect the varying ethnic groups, selection criteria, and the number of cases included in different studies. 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 pre-eclampsia 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. Pre-eclampsia, 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 χ² 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 χ² (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. Pre-eclampsia 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/mm³ 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 means±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 (homozygous) | 16 | 12 | 1.3 (0.6-2.6) | 0.271 |
| 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 |

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. 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. Lindqvist et al. found no difference in prevalence for this mutation in preeclampsia or IUGR patients. 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.

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. 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.

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. Driul et al. 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. In our patients the frequency of homozygosity for the cytosine 677 thymine (C677T) mutation in MTHFR has been reported previously by several groups, but has not been found in other studies. In our patients the frequency of 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 prothrombin gene mutation in MTHFR has been reported previously by several groups, but has not been found in other studies. In our patients the frequency of 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).

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. 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 (homozygous), MTHFR mutation (homozygous), and deficiencies of protein S, protein C, and antithrombin III were not statistically significant in the study group compared with the control group. As a result, we do not recommend screening for thrombophilia in hypertensive disorders of pregnancy in our population.
V Leiden-mutation, coagulation inhibitor deficiency, and elevated antiphospholipid-antibodies.

Kunzmann e, Hommel G. Incidence of the factor 7.

Thromb Res. 2000; 100:363-5.

Hemolysis, elevated liver-enzymes, low platelets.

American College of Obstetricians and Gynecologists, Washington DC (1996).

References

1. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet 2001; 357:53-6.
2. British Committee for Standards in Haematology (1999) Guidelines on investigation and management of thrombophilia. Journal of Clinical Pathology. 1999; 42:702-9.
3. Many A, Eldad R, Yaron Y, Eldor A, Lessing JB, Kupferminc MJ. Third-trimester unexplained intrauterine fetal death is associated with inherited thrombophilia. Obstet Gynecol 2002;99:884-7.
4. Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavidia A, Agrarastos T, Zournati V, Makris PE, Bontis J, Kotis A: Factor V leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod 2000; 15:456-62.
5. Heflari L, Jirecsek S, Heim K, Grimm C, Antenstein-er G, Zeilinger R, Husslein P, Tempfer C. Genetic polymorphisms associated with thrombophilia and vascular disease in women with unexplained late intrauterine fetal death: a multicenter study. J Soc Gynecol Invest. 2004; 11:42-4.
6. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing JB. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999; 340:9-13.
7. Von Tempelhoff GF, Heilmann L, Spanuth E, Kunzmann E, Hommel G. Incidence of the factor V Leiden-mutation, coagulation inhibitor deficiency, and elevated antiphospholipid-antibodies in patients with preeclampsia or HELLP-syndrome. Hemolysis, elevated liver-enzymes, low platelets. Thromb Res. 2000; 100:363-5.
8. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. ACOG technical bulletin no. 219. American College of Obstetricians and Gynecologists, Washington DC (1996).
9. Brown MA, Hague WM, Higgins J, Lowe S, McCowan L, Oats J, Peak MJ, Rowan JA, Walters BN. The detection, investigation and management of hypertension in pregnancy. Aust N Z J Obstet Gynaecol 2000; 40:139-55.
10. Kupferminc MJ, Fait G, Many A, Gordon D, El- dor A, Lessing JB: Severe preeclampsia and high frequency of genetic thrombophilic mutations. Obstet Gynecol 2000; 96: 45-49.
11. Lindqvist PG, Svensson P, Dahliback B, Mar- sal K: Factor V Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss a possible evolutionary selection mechanism. Thromb Haemost 1998;79: 89-73.
12. Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM. Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM. Maternal and fetal inherited thrombophilia are not related to the development of severe preeclampsia. Am J Obstet Gynecol 2001; 185:153-7.
13. Grandone E, Margaglione M, Colaizzo D, Cap- pucci G, Scianname N, Montanaro S, Paladini D, Martinei P, Di Minno G. Prothrombotic genetic risk factors and the occurrence of gestational hypertension with or without proteinuria. Thromb Haemost 1999; 81:349-52.
14. Sehbo S, Arimani T, Hamada H, Yamada N, Hamaguchi K, Kudo T. Methyleneetetrahydrofolate reductase polymorphism and preeclampsia. J Med Genet 1997; 34:525-6.
15. O’Shaughnessy K, Fu B, Ferraro F, Lewis I, Downing S, Morris NH. Factor V Leiden and thrombomielin tetrahydrofolate reductase gene variants in an East Anglian preeclampsia cohort. Hypertension 1999;33:1338-41.
16. Davalos IP, Moran MC, Martinez-Abundis E, Gonzalez-Ortiz M, Flores-Martinez SE, Machorro V, Sandoval L, Figuera LE, Mensa JP, Olioma JM, Tlacuilo-Parra JA, Sanchez-Corona J, Salazar-Paramo M. Methyleneetetrahydrofolate reductase C677T polymorphism and Factor V Leiden variant in Mexican women with preeclampsia/eclampsia. Blood Cells Mol Dis. 2005; 34:125-6.
17. Sayin M, Varol FG, Sayin NC. Evaluation of natural coagulation inhibitor levels in various hypertensive states of pregnancy. Eur J Obstet Gynecol Reprod Biol. 2005; [Epub ahead of print].
18. Sayin M, Varol FG, Sayin NC. Evaluation of natural coagulation inhibitor levels in various hypertensive states of pregnancy. Eur J Obstet Gynecol Reprod Biol. 2005; [Epub ahead of print].