TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 1094-1100

doi:10.3906/sag-1502-132

Coexistence of preeclampsia and inherited thrombophilia in Turkish pregnant women

Mehtap POLAT[1,*], Ebru Hacer BİBEROĞLU[2], İsmail GÜLER[3], Ömer Kutay BİBEROĞLU[4]

[1]Anatolia Women and IVF Health Center, Ankara, Turkey
[2]Dr. Zekai Tahir Burak Women Health Care, Education and Research Hospital, High Risk Pregnancy Unit, Ankara, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Gazi University, Ankara, Turkey
[4]Department of Obstetrics and Gynecology, Gynecological Endocrinology Unit, Faculty of Medicine, Gazi University, Ankara, Turkey

Received: 23.02.2015 • **Accepted/Published Online:** 10.10.2015 • **Final Version:** 23.06.2016

Background/aim: To examine the relationship of inherited thrombophilia and other thrombotic risk factors with preeclampsia (PE) in a population of pregnant Turkish women.

Materials and methods: This was a case cross-sectional study in which 70 women with PE and 60 normal pregnant women were studied to find out the frequency of women with risk factors including inherited thrombophilia among preeclamptic cases.

Results: Hemoglobin, platelet count, uric acid, vitamin B12, folic acid, copper, homocysteine, plasminogen activator inhibitor-1, fibrinogen, protein S, protein C, activated protein C resistance values show significant differences in women with PE in comparison to women with normal pregnancy.

Conclusion: There may be a link between inherited thrombophilia and PE, at least in a sample of Turkish pregnant women. We also propose that the association between thrombophilia and PE is stronger than suggested previously. Furthermore, copper is selectively elevated in women with PE as an independent marker.

Key words: Copper, homocysteine, plasminogen activator inhibitor-1, protein S, activated protein C resistance

1. Introduction

Preeclampsia (PE) is a disorder of pregnancy associated with widespread vascular endothelial malfunction and vasospasm. The pathophysiology of PE likely involves both maternal and fetal/placental factors. Attention has been focused on the link between inherited thrombophilia and PE. The results of previous studies are controversial, with some confirming (1–3) and others denying the link between the two (4–6).

The heterogeneity among study results is undeniable. Very few studies have mentioned the possible influence of confounders such as ethnicity (7,8).

There is considerable evidence concerning trace elements of serum level changes associated with PE. Copper (Cu) is an essential cofactor for the enzymes catalase, superoxide dismutase, and cytochrome oxidase, and its deficiency can lead to a variety of vascular and nutritional disorders (9). There are studies showing higher (9), similar (10), or even lower levels of Cu (11) in the circulation of women with PE in comparison to healthy pregnant women.

The objective of this study was to examine the relationship between inherited thrombophilia and other thrombotic risk factors and PE in a population of pregnant Turkish women.

2. Materials and methods

One hundred and thirty pregnant women who presented to Gazi University

Medical School's Department of Obstetrics between October 2002 and November 2004 were categorized in the third trimester of their pregnancies into 2 groups. Group 1 and group 2 included 70 women with preeclampsia (group 1: study group) and 60 normotensive pregnant women (group 2: control group), respectively. The clinical data and blood samples were collected prospectively, but laboratory work-up and data evaluation were achieved retrospectively, without any intervention. Universal principles of the Helsinki Declaration were applied in this nonbiomedical case control study.

The inclusion criteria were nulliparity and having a normal 50-g glucose loading test for both groups. Blood

* Correspondence: mehtappolat1977@yahoo.com

pressure measurement equal to or more than 140/90 mmHg (at least twice over a 6-h period), and proteinuria (more than 0.3 g/24 h or more than +2 protein in a spot urine test) were additional requirements for group 1.

Multiparous women and women with preexisting essential hypertension, diabetes mellitus, liver, kidney or metabolic diseases, any history of current drug use excluding iron supplements, excessive physical activity, a history of recurrent miscarriage, intrauterine fetus demise, or personal and family history of thromboembolic disease were excluded from both groups.

Regarding clinical follow up, all women were examined every 2 to 3 weeks between 28 and 36 weeks and weekly thereafter, but the optimal frequency and timing of prenatal care visits especially in the PE group were determined according to the needs and risk status of each woman and her fetus. Additional ultrasound examinations following a routine one performed between 18 and 22 weeks were done according to the presence of maternal or fetal indications. Whenever the health of the mother and the fetus were endangered by severe PE, induction of labor and early delivery, either vaginally or by cesarean section, was achieved.

Blood samples were taken upon admission to hospital following 6 h of fasting for the following measurements: hemoglobin (Hb), hematocrit (Htc), platelet count (PLT), serum albumin (Alb), uric acid (UAC), creatinine (CR), Cu, homocysteine (Hcy), vitamin B12 (vit B12), folic acid (FOA), fibrinogen (FBN), protein C (PC), protein S (PS), antithrombin III (AT III), activated protein C resistance (aPCR), and plasminogen activator inhibitor-1 (PAI-1). All tests were carried out at the hospital's central laboratory, except thrombophilia markers. *Atomic absorption* spectrometry (AA 6701F Atomic Absorption Flame Emission Spectrophotometer, Shimadzu, Japan) was used to measure Cu, and high performance liquid chromatography (*HPLC*) (Waters, Germany) was used to measure *Hcy*.

For thrombophilia tests, 10 mL of blood was placed in EDTA vacutainer tubes, kept on ice and centrifuged at 2500 rpm for 10–30 min within 30 min of the sample being drawn, and the plasma was carefully extracted and frozen to –70 °C and then transferred to the hospital laboratory on dry ice. The sample tubes were numbered without any patient identification so that the laboratory could be blinded. Thrombophilia markers were investigated in the hospital's central genetics laboratory. The STA - Staclot aPCR kit (Diagnostica Stago, Asnieres, France) was used for the assessment of the aPCR in plasma. Plasma samples whose clotting times were ≥120 s or <120 s were considered aPCR negative or aPCR positive, respectively (8). Asserachrom (Diagnostica Stago, Asnieres, France) ELISA kit for PAI-1, STA LIATEST immunoturbidimetric assay kit (Diagnostica Stago, Asnieres, France) for protein C & S, and *STA* Antithrombin *III assay* were used.

The statistical analyses were conducted with the Statistical Package for the Social Sciences (SPSS/PC-11) (SPSS Inc. Chicago, IL, USA). All data were provided on an average ± standard deviation basis. The statistical analysis of the differences between the patient and control groups for the parameters showing normal distribution (PC, aPCR, PAI-1) was done with a t-test. The Mann–Whitney U test was used to make comparisons among the parameters that did not demonstrate normal distribution. The mean values of Hcy, Cu, PAI-1, PS, PC, AT III, and aPCR in preeclamptic blood samples were defined as abnormally high or as abnormally low if the measured values were more than 95% or less than 5% of the average levels measured in the control group, respectively. The groups were then compared using Pearson's chi-square test. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

PE (group 1) and normotensive control (group 2) pregnant women had similar ages and comparable smoking and Turkish coffee drinking habits. Body weight and BMI values were higher in PE women than in the control group (Table 1). Further, PE women had higher average systolic/diastolic blood pressure measurements as expected, shorter duration of pregnancy, lower birth weight, and newborn Apgar scores when compared with the control group (Table 2). Out of the 70 PE women, 19 (27%) revealed intrauterine growth restriction (IUGR) fetuses, an additional 2 (2.85%) had intrauterine ex fetuses, and 2 (2.85%) had placental abruption (PLABR). Such complications were not encountered in any of the control cases. Labor had to be induced more frequently in the PE group. Cesarean section rather than vaginal delivery was the preferred mode of delivery in both groups (Table 3). Regarding the laboratory results, PC, vit B12, and FOA levels were lower and Hb, UAC, and Cu values were higher in Group 1 as compared to Group 2. The mean values of Htc, Alb, and CR were similar between the groups (Table 4). In terms of coagulation factors, PAI-1, aPCR, Hcy, and FBN values were higher and mean PS values were lower in the PE patients when compared to the normal control group (Table 5). Table 6 reveals the cut-off levels of each parameter and the percentage of women with abnormal test results in the PE and control groups. As seen from the data, Cu was elevated in 85.7%, PAI-1 in 77.1%, aPCR in 31.4%, and Hcy in 28.6% of the patients in the PE group, while PS was suppressed in 52.9%. In Table 7, relative risk ratios of developing PE with abnormal thrombophilia markers and Hcy and Cu blood levels (odds ratios and 95% confidence intervals) are demonstrated.

Table 1. Epidemiological characteristics of women in preeclamptic and control groups.

Parameter	Preeclampsia(Gr I)	Control (Gr II)	P
Women (n)	70	60	
Age (year)	28.92 ± 4.71	29.4 ± 3.48	>0.05
Weight (kg)*	70.6 ± 10.74	65.9 ± 5.32	<0.01
BMI (kg/m^2)*	26.79 ± 4.42	24.83 ± 1.67	<0.01
Nonsmoker	39 (56%)	36 (60%)	>0.05
Smoker < 10/ day	5 (7%)	3 (5%)	>0.05
Smoker ≥ 10/ day	26 (37%)	21 (35%)	>0.05
Non-coffee drinker	37 (53%)	32 (53%)	>0.05
Turkish coffee 1 cup/day	5 (7%)	3 (5%)	>0.05
Turkish coffee ≥2 cup/day	28 (40%)	25 (42%)	>0.05

BMI: Body mass index

Table 2. Clinical characteristics of women in preeclamptic and control groups (mean±SD).

Parameter	Preeclampsia(Gr I)	Control (Gr II)	P
Women (n)	70	60	
Systolic BP (mmHg)*	162 ± 12.17	106 ± 17.38	<0.01
Diastolic BP(mmHg)*	99 ± 12.17	67 ± 8.89	<0.01
Duration of Pregnancy(wk)*	34.2 ± 2.64	37.3 ± 2.26	<0.01
Birth weight (gr) *	2615 ± 667.9	3128 ± 325.9	<0.01
Apgar (1. minute)*	8 ± 1.44	9 ± 0.37	<0.05
Apgar (5. minute)*	8 ± 1.44	9 ± 0.37	<0.05

BP: Blood pressure

Table 3. Labor characteristics and mode of delivery in women with preeclampsia and control groups.

Parameter	Preeclampsia(Gr I)		Control (Gr II)		P
	n	%		%	
Women (n)	70		60		
Induction of labor*	13	18.6	2	3.3	<0.001*
Vaginal delivery	20	28.6	24	40	0.141
Cesarean delivery	50	71.4	36	60	0.141

4. Discussion

In our study, all of the pregnant women in both the PE and control groups were young and nulliparous with single fetuses, without individual risk factors like personal history of chronic hypertension, diabetes mellitus, renal or any autoimmune diseases, or family history of PE.

Table 4. Laboratory parameters of women in preeclamptic and control groups (mean±SD).

Parameter	Preeclampsia(Gr I)	Control (Gr II)	P
Women (n)	70	60	
Hemoglobin (gr/dl)	11.79 ± 1.35	11.48 ± 1.47	0.013
Hematocrit (%)	34.99 ± 3.28	35.04 ± 3.10	0.842
Platelet/µL	198.421 ± 61.276	224.416 ±53.397	0.002
Albumin (mg/dl)	3.36 ± 0.43	3.44 ± 0.19	0.292
Creatinine (mg/dl)	0.68 ± 0.11	0.70 ± 0.09	0.181
Uric acid (mg/dl)	3.79 ± 1.42	3.06 ± 0.38	0.001
Vit. B12 (pg/ml)	223.83 ± 95.15	276.83 ± 69.80	0.001
Folic acid (ng/ml)	10.53 ± 4.11	11.32 ± 5.40	0.017
Cupper (µgr/dl)	210.95 ± 56.58	121.21 ± 25.08	0.001

Table 5. Thrombophilia markers and serum homocysteine levels in women with preeclampsia and control groups (mean±SD).

Parameter	Preeclampsia(Gr I)	Control (Gr II)	P
Women (n)	70	60	
Fibrinogen (mg/dl)	422.28 ± 85.49	327.35 ± 66.63	<0.001
Homocysteine (µmol/L)	10.5 ± 3.6	7.25 ± 2.44	<0.001
Protein C (%)	101.58 ± 18.33	109.70 ± 16.80	0.825
Protein S (%)	55.55 ± 28.9	68.48 ± 17.22	<0.001
Antithrombin III (%)	87.14 ± 21.46	90.15 ± 13.08	0.740
PAI-1 (ng/dl)	84.06 ± 32.35	30.53 ± 12.14	<0.001
APCR (sec) (pg/ml)	121.39 ± 33.17	99.22 ± 20.90	<0.001

aPCR: Activated protein C resistance

Table 6. The cut off levels and the percentage of women with abnormal test results in the preeclamptic and control patient groups.

Parameter		Preeclampsia(Gr I)		Control (Gr II)		P
		n	%	n	%	
Women (n)		70		60		
Protein S (%)	<48%	37	52.9	2	3.3	<0.001
	>48%	33	47.1	58	94.7	<0.001
PAI-1 (ng/dl)	<58.37	16	22.9	57	95	<0.001
	>58.37	54	77.1	3	5	<0.001
APCR (sec)	<138.48	48	68.6	57	95	<0.001
	>138.48	22	31.4	3	5	<0.001
Homocysteine(µmol/L)	<10.97	50	71.4	57	95	<0.001
	>10.97	20	28.6	3	5	<0.001
Cupper (µgr/dl)	<159.80	10	14.3	57	95	<0.001
	>159.80	60	85.7	3	5	<0.001

aPCR: Activated protein C resistance; PAI-1: Plasminogen activator inhibitor-1

Table 7. Likelihood ratios of developing preeclampsia with abnormal thrombophilia markers, homocysteine and copper blood levels.

Parameter	Odds ratio (OR)	Confidence interval (CI)
PAI-1	64.12	17.6 - 232.5
Protein S	32.51	7.36 -143.64
APCR	8.7	2.4- 30.88
Homocysteine	7.6	2.1- 27.1
Cupper	114	29.8- 435.4

aPCR: Activated protein C resistance; PAI-1: Plasminogen activator inhibitor-1

This enabled us to study a well-selected low-risk group of pregnant women and to minimize the confounding factors other than thrombophilia, which might increase the risk of PE occurring.

The mean maternal age in both the PE and the control groups was similar, supporting the view that age was not a significant factor in the development of PE, in accordance with some studies (12) but in contrast to others (13). It has been argued that current evidence on the association between maternal age and perinatal outcome remains largely clouded by age-related confounding factors (14). High maternal BMI is associated with several pregnancy-related complications including PE, presumably related to the presence of insulin resistance and associated endothelial disorder (15). Our PE patients were not obese but more overweight than the control group. It has long been known that environmental influences, such as smoking, alcohol use, poor nutrition, and thrombophilic state leading to placental thrombosis have causative roles, not only in the development of PE, but also in IUGR (16,17). We recognize that each or both of thrombophilia and PE, by causing intervillous thrombosis, and consequent placental perfusion impairment, could be the reason behind IUGR. This cascade may eventually result in intrauterine fetal demise and/or PLABR. In accordance with the literature, we found that out of 70 PE women, 19 (27%) revealed IUGR fetuses, an additional 2 (2.85%) had intrauterine ex fetuses, and 2 (2.85%) had PLABR. Such complications were not encountered in any of our control cases. The newborns of the PE women in the present study were born more prematurely, with lower birth weight and with lower Apgar scores when compared to our control group.

An elevation in Hb or Htc is hypothesized to be the result of a combination of reduced plasma circulating volume and enhanced erythropoiesis because of underlying placental hypoxia. Thrombocytopenia also accompanies PE and its severity is generally parallel to that of the underlying disease (18). In our study, PE women had significantly higher Hb values and lower PLT counts than the women in the control group. Although hyperuricemia (19) and low Alb levels (20) are usually considered significant indicators of severity of PE and poor prognosis of perinatal outcome, our PE patients as compared to controls had similar normal serum levels of Alb and CR but significantly higher serum levels of UAC. Although serum UAC and CR are expected to be strongly interrelated, not all studies demonstrated both to be elevated (21), including ours, perhaps due to the fact that serum CR may be a less sensitive marker of hypertensive disease in pregnancy.

In the present study, we demonstrated that the serum Cu levels were significantly elevated in PE versus normal pregnant women, so much so that more than 85% of PE women revealed levels above the threshold value. The likelihood ratio of developing PE in women with elevated Cu levels was 114, being the highest among the parameters we measured including thrombophilia markers. Although this finding is very intriguing, it has to be reproduced by other studies.

Hyperhomocysteinemia has been reported in PE women (22). Our PE patients also revealed higher Hcy and lower FOA and B12 levels in accordance with previous studies. The presence of high Hcy values increased the relative risk of PE by 1.32–3.2, while the RR reached 9.7 in primiparas and 6.9 in obese patients (23). In the present study, in primiparas, the likelihood of development of PE was found to be 7.6 times higher in women with elevated Hcy serum levels, which were detected in 28.6% of our PE cases. Furthermore, FBN, Hcy, PAI-1, and aPCR values were higher and PS values lower in women with PE in comparison to control group 2. The likelihood ratios of developing PE with abnormal blood levels of thrombophilia markers and the percentages of women with abnormal test results in women with PE were OR 64.12 and 77.1% for PAI-1, OR 32.51 and 47.1% for PS, OR 8.7 and 31.4% for aPCR, and OR 7.6 and 28.6% for Hcy,

respectively (Tables 6 and 7). Among all the parameters we studied, serum Cu levels predicted PE the most with an odds ratio of 114, which was found to be elevated in more than 85% of PE women. Since mutations in the factor V mutations are related to aPCR and FVL is reported in about 90% of patients with aPCR in the general population (24), we chose to measure aPCR as a marker. In contrast to some previous studies (25,26), we did not find an increased frequency of suppressed PC and AT III levels in women with PE as compared to control women.

Several recent meta-analyses that have attempted to analyze the collective data have reported that pooled OR for associations between inherited thrombophilia and PE are generally in the 1.0 to 2.5 range, indicating that thrombophilia may be only weakly associated with PE, if at all (26,27). Based on our results, we propose that the association between thrombophilia, especially through studying PAI-1 and PS markers, and PE is stronger than suggested by the majority of publications in the medical literature. Most important of all, Cu as an independent marker is selectively and remarkably elevated in PE women in late pregnancy as compared to normal pregnant women. If this finding can be reproduced by others and if serum Cu levels can be demonstrated to begin increasing in the first trimester before the symptoms and findings of PE exist, Cu could be a valuable tool to predict PE and perhaps other complications of pregnancy.

Our study had some limitations such as being done in a tertiary center, thereby not reflecting the general Turkish population, and the small number of patients enrolled. One of the strengths is that potential confounding factors such as age, ethnicity, and systemic medical problems were overcome by the homogeneity of this study cohort and the participation of only primigravid women with no history of miscarriage.

Whether the association between thrombophilia and PE is causal or temporal, and more specifically whether thrombophilia acts as a cofactor in the pathogenesis of PE or accelerates its course, is speculative. Lastly, our data should not be considered as a justification for screening for thrombophilia in normo- or hypertensive pregnant women since there is no clear evidence that anticoagulation can prevent PE or improve the outcome.

Acknowledgment
This work was supported by grants for research scientific projects from Gazi University, Ankara, Turkey.

References

1. Lykke JA, Bare LA, Olsen J, Lagier R, Arellano AR, Tong C, Paidas MJ, Langhoof-Roos J. Thrombophilias and adverse pregnancy outcomes: results from the Danish National Birth Cohort. *J Thromb Haemost* 2012; 10: 1320-1325.
2. Reyes LM, García RG, Ruiz SL, Camacho PA, Ospina MB, Aroca G, Accini JL, López-Jaramillo P. Risk factors for preeclampsia in women from Colombia: a case-control study. PLoS One 2012; 7: e41622.
3. Kupferminc MJ, Fait G, Many A, Gordon D, Eldor A, Lessing JB. Severe preeclampsia and high frequency of genetic thrombophilic mutations. *Obstet Gynecol* 2000; 96: 45-49.
4. Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM. Maternal and fetal inherited thrombophilias are not related to the development of severe preeclampsia. *Am J Obstet Gynecol* 2001; 185: 153-157.
5. Kahn SR, Platt R, McNamara H, Rozen R, Chen MF, Genest J Jr, Goulet L, Lydon J, Seguin L, Dassa C et al. Inherited thrombophilia and preeclampsia within a multicenter cohort: the Montreal Preeclampsia Study. *Am J Obstet Gynecol* 2009; 200: *151*.e1-151.*e9*.
6. Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, Seligsohn U, Carrier M, Solomon O, Greer IA. The association of factor V Leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: a systematic review and meta-analysis of prospective cohort studies. *PLoS Med* 2010; 7: e1000292.
7. Rasmussen A, Ravn P. High frequency of congenital thrombophilia in women with pathological pregnancies? Acta Obstet Gynecol Scand 2004; 83: 808-817.
8. Kist WJ, Janssen NG, Kalk JJ, Hague WM, Dekker GA, de Vries JI. Thrombophilias and adverse pregnancy outcome - A confounded problem! Thromb Haemost 2008; 99: 77-85.
9. Katz O, Paz-Tal O, Lazer T, Aricha-Tamir B, Mazor M, Wiznitzer A, Sheiner E. Severe preeclampsia is associated with abnormal trace elements concentrations in maternal and fetal blood. J Matern Fetal Neonatal Med 2012; 25: 1127-1130.
10. Fenzl V, Flegar-Meštrić Z, Perkov S, Andrišić L, Tatzber F, Žarković N, Duić Ž. Trace elements and oxidative stress in hypertensive disorders of pregnancy. Arch Gynecol Obstet 2013; 287: 19-24.
11. Sarwar MD, Ahmed S, Ullah MS, Kabir H, Rahman GKM, Safiqul Islam HAM. Comparative study of serum zinc, copper, manganese, and iron in preeclamptic pregnant women. Biol Trace Elem Res 2013; 154: 14-20.
12. Ohkuchi A, Iwasaki R, Suzuki H, Hirashima C, Takahashi K, Usui R, Matsubara S, Minakami H, Suzuki M. Normal and high-normal blood pressures, but not body mass index, are risk factors for the subsequent occurrence of both preeclampsia and gestational hypertension: a retrospective cohort study. Hypertens Res 2006; 29: 161-167.

13. Khalil A, Syngelaki A, Maiz N, Zinevich Y, Nicolaides KH. Maternal age and adverse pregnancy outcomes: a cohort study. Ultrasound Obstet Gynecol 2013; 42: 634-643.

14. Ales KL, Druzin ML, Santini DL. Impact of advanced maternal age on the outcome of pregnancy. Surg Gynecol Obstet 1990; 171: 209-216.

15. Mbah AK, Kornosky JL, Kristensen S, August EM, Alio AP, Marty PJ, Belogolovkin V, Bruder K, Salihu HM. Super-obesity and risk for early and late preeclampsia. BJOG 2010; 117: 997-1004.

16. 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.

17. Franchi F, Cetin I, Todros T, Antonazzo P, Nobile de Santis MS, Cardaropoli S, Bucciarelli P, Biguzzi E. Intrauterine growth restriction and genetic predisposition to thrombophilia. Haematologica 2004; 89: 444-449.

18. Troeger C, Holzgreve W, Ladewig A, Zhong XY, Hahn S. Examination of maternal plasma erythropoietin and activin A concentrations with regard to circulatory erythroblast levels in normal and pre-eclamptic pregnancies. Fetal Diagn Ther 2006; 21: 56-60.

19. Kocijancic DM, Plesinac S, Plecas D, Aksam S, Kocijancic A. Correlation of biochemical parameters and neonatal outcome in patients with gestational hypertension. Clin Exp Hypertens 2013; 35: 6-10.

20. Seong WJ, Chong GO, Hong DG, Lee TH, Lee YS, Cho YL, Chun SS, Park IS. Clinical significance of serum albumin level in pregnancy-related hypertension. J Obstet Gynaecol Res 2010; 36: 1165-1173.

21. Hawkins TL, Roberts JM, Mangos GJ, Davis GK, Roberts LM, Brown MA. Plasma uric acid remains a marker of poor outcome in hypertensive pregnancy: a retrospective cohort study. BJOG 2012; 119: 484-492.

22. Acilmis YG, Dikensoy E, Kutlar AI, Balat O, Cebesoy FB, Ozturk E, Cicek H, Pence S. Homocysteine, folic acid and vitamin B12 levels in maternal and umbilical cord plasma and homocysteine levels in placenta in pregnant women with pre-eclampsia. J Obstet Gynaecol Res 2011; 37: 45-50.

23. Dekker AG, Morris NH. Medical conditions associated with hypertensive disorders of pregnancy. In: Sibai BM, editor. Hypertensive Disorders in Women. Philadelphia, PA, USA: W.B. Saunders; 2001. pp. 85-110.

24. Williamson D, Brown K, Luddington R, Baglin C, Baglin T. Factor V cambridge: a new mutation (Arg306→Thr) associated with resistance to activated protein C. Blood 1998; 91: 1140-1144.

25. Giovanni L, Antonio AP, Danilo C, Stefano G, Therese D, Elisabetta RM, Letizia B, Elio C, Domenico A. Thrombophilias and pregnancy complications: a case-control study. Int J Biomed Sci 2007; 3: 168-175.

26. Larciprete G, Gioia S, Angelucci PA, Brosio F, Barbati G, Angelucci GP, Frigo MG, Baiocco F, Romanini ME, Arduini D et al. Single inherited thrombophilias and adverse pregnancy outcomes. J Obstet Gynaecol Res 2007; 33: 423-430.

27. Robertson L, Wu O, Langhorne P, Twaddle S, Clark P, Lowe GD, Walker ID, Greaves M, Brenkel I, Regan L et al. Thrombophilia in pregnancy: a systematic review. Br J Haematol 2006; 132: 171-196.