INHERITED THROMBOPHILIA IN TURKISH WOMEN, AS A RISK FACTOR FOR ISCHEMIC PLACENTAL DISEASES

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Abstract

We aim to elucidate the link between inherited thrombophilias and pregnancies complicated with ischemic placental diseases, in Turkish women. This is a case control study comprising 140 women with ischemic placental disease and 60 uncomplicated pregnant women. Parameters including haemoglobin, platelet count, uric acid, vitamin B12, folic acid, copper, homocysteine, plasminogen activator inhibitor-1, fibrinogen, protein S, protein C, activated protein C resistance values were significantly changed in women with ischemic placental disease when compared to those with normal pregnancies.

The association between thrombophilia and ischemic pregnancy diseases is stronger than suggested previously. Furthermore, copper is selectively elevated in complicated pregnancies.

Keywords: Pregnancy, ischemic pregnancy diseases, thrombophilia

Introduction

It has been suggested that maternal inherited thrombophilia may be associated with complications of pregnancy resulting in adverse outcomes (Pobinger I, 2005) based on studies, one of which reported that 65% of women with preeclampsia, intrauterine growth restriction, unexplained intrauterine fetal demise or placental abruption had thrombophilia with an odds ratio of 5.2% for some form of mutation (Kupferming, 1999). Consequences of thrombophilia including interference with trophoblast

differentiation, inadequate placentation or thrombosis of placental vasculature might be the factor triggering the biological and clinical manifestations of ischemic placental diseases (Kupferming, 1999; Kujovich, 2004). The paper aims at to examine the magnitude of the association between inherited thrombophilia and pregnancy complicated with ischemic placental diseases (IPL) including preeclampsia (PE), intrauterine growth restriction (IUGR) and intrauterine fetal demise (IUFD).

Material and methods

Two hundred pregnant women who applied to Gazi University Medical School, Department of Obstetrics between October 2002 and November 2004 were categorized in the third trimester of their pregnancies into 3 groups. Group 1, group 2 and group 3 included 70 women with preeclampsia (PE) of whom 19 had IUGR, 2 had PLABR and 2 had IUFD, 70 women normotensive but with other ischemic placental diseases (IPD) consisting of 48 cases of intrauterine growth restriction (IUGR) and 22 cases with intrauterine fetal demise (IUFD) of whom 3 patients also had abruptio placenta, and 60 normotensive normal pregnant controls without any complications, respectively.

All women were personally informed about the objectives of the study and permission was taken from the local institutional committee. The clinical data and blood samples were collected prospectively, but laboratory workup and data evaluation were achieved retrospectively, without any intervention. Universal principles of Helsinki Declaration were applied in this non-biomedical study.

The inclusion criteria were nulliparity and having normal 50-gr glucose loading test, for all 3 groups. Having blood pressure equal or more than 140 / 90 mmHg (at least twice over a 6 hour interval), proteinuria (more than 0.3 gr / 24 hours or more than +2 protein in a spot urine test) for group 1 and ischemic placental disease other than PE for group 2. The exclusion criteria were multiparity and having preexisting essential hypertension, diabetes mellitus, liver, kidney or metabolic diseases, any history of current drug use excluding iron supplement, excessive physical activity, a history of recurrent miscarriage, personal and family history of thromboembolic disease for all of the cases.

In the normotensive pregnancy group, the women were examined every 2 to 3 weeks between 28 and 36 weeks and weekly thereafter, whereas the optimal frequency, timing of prenatal care visits and methods of evaluation including fetal movement counting, nonstress testing, biophysical profile assessment and doppler flow studies were carried out 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 maternal or fetal indications. When severe PE, IUGR, IUFD or PLABR, induction of labor and early delivery, endangered health of the mother and the fetus either vaginally or by cesarean section was achieved.

Blood samples were taken upon admission to the hospital following 6 hours of fasting for the following measurements; hemoglobin (Hb), hematocrit (Htc), platelet count (PLT), serum albumin (Alb), uric acid (UAC), creatinine (CR), copper (Cu), homocysteine (Hcy), vitamin B12 (vit B12), folic acid (FOA), fibrinogen (FBN), protein C (PC), protein S (PS), antitrombin III (AT III), activated protein C resistance (aPCR) and plasminogen activator inhibitor-1 (PAI-1). All tests were carried out at the central laboratory of the hospital except thrombophilia markers. Atomic Absorption Spectrometry to measure Cu and high performance liquid chromatography (HPLC) to measure Hcy was used.

For thrombophilia tests, 10 ml of whole blood was placed in EDTA vacutainer tubes, kept on ice and centrifuged at 2500 rpm for 10-30 minutes within 30 minutes, plasma carefully extracted and frozen to -70 °C and transferred to the laboratory of the hospital on dry ice. The sample tubes were just numbered without any patient identification so that the laboratory could be blinded. Thrombophilia markers were investigated in the hospital's central genetic laboratory. The STA® - Staclot® APC-R kit was used for the assessment of the activated protein C resistance (aPC-R) in plasma. Plasma samples whose clotting times were $\geq$ 120 seconds, or < 120 seconds were considered to be aPCR negative, or aPCR positive respectively. Asserachrom (Diagnostica Stago, France) Elisa kit for PAI-1, STA LIATEST immunoturbidimetric assay kit for protein C & S, 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 Ill, and USA. All data was provided on an average $\pm$ 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 one sided variance analysis "ANOVA". Multiple comparisons were carried out to distinguish the differences in the groups of the substantive data from the ANOVA one sided variance analysis by using the "Bonferroni test". The "Kruskal-Wallis variance analysis" was used to make comparisons among the parameters that did not demonstrate normal distribution. When significant differences were found in parameters as a result of the Kruskal-Wallis variance analysis, the WJ Canover Practical Nonparametric test was used to which group is different from the other group? The mean values of Hcy, Cu, PAI-1, PS, PC, AT III and aPCR in preeclamptic women blood samples are defined as abnormally high or as abnormally low if the measured

values are more than %95 or less than %5 of the average levels measured in the control group women respectively. The groups were then compared with the Pearson Chi-Square test. A value of $p<0.05$ was considered as statistically significant.

Results

Pregnant women with PE (group I), other IPD (group 2) and normotensive control (group 3) were at similar ages. While body weight and BMI values were higher in preeclamptics than in the control group ($p = 0.016$). They were not different in-group 2 when compared to groups 1 & 3. The numbers of women who are smoking and / or drinking Turkish coffee were similar in all groups. **(Table 1)**

Table 1. Epidemiological characteristics of women with preeclampsia, other ischemic placental diseases and control groups (mean ± SD)

Parameter	Preeclamptic pregnancies (Gr I)	Other complications (Gr II)	Normal controls (Gr III)
Women (n)	70	70	60
Age (year)	28.9 ± 4.71	28.4 ± 4.83	29.4 ± 3.48
Weight (kg)†	70.6 ± 10.74*	68.1 ± 9.13	65.9 ± 5.32*
BMI (kg/m²)†	26.7 ± 4.42*	25.9 ± 3.48	24.8 ± 1.67*
Nonsmoker	39 (% 56)	41 (% 59)	36 (% 60)
Smoker <10/d	5 (% 7)	4 (% 6)	3 (% 5)
Smoker ≥10/d	26 (% 37)	25 (% 36)	21 (% 35)
Non-coffee drinker	37 (% 53)	40 (% 58)	32 (% 53)
Turkish coffee 1 cup/d	5 (% 7)	7 (% 10)	3 (% 5)
Turkish coffee ≥2cup/d	28 (% 40)	23 (% 33)	25 (% 42)

*P = 0.0016 - † difference between Gr's 1 & 3)

Further, women with PE (group 1) had higher average systolic / diastolic blood pressure measurements than groups 2 & 3, as expected ($p = 0.001$). The duration of pregnancy in women with IPD (groups 1 & 2) was shorter (median of 34.2 and 35.4 weeks, $p = 0.018$ and $p= 0.001$, respectively) as opposed to group 3. Women with PE (group 1) also delivered earlier than women with IPD (group 2) ($p = 0.003$). Birth weights and newborn Apgar scores were also lower in groups 1 & 2 in comparison to group 3 and also in-group 2 in comparison to group 1 & 3 ($p = 0.001$ within all groups). In other words, we encountered the lowest birth weight and

apgar scores in-group 2, which is an expected finding since this group included also the women with IUFD. **(Table 2)**

Table 2. Clinical characteristics of women with preeclampsia, other ischemic placental diseases and normal control groups (mean ± SD)

Parameter	Preeclamptic pregnancies (Gr I)	Other complications (Gr II)	Normal controls (Gr III)
Women (n)	70	70	60
Systolic BP (mmHg)†	162* ±12.17	105* ±14.69	106* ±17.38
Diastolic BP (mmHg)†	99* ±12.17	65* ± 8.77	67* ± 8.89
Gestational age (wks)↑	34.2* ± 2.64	35.4*± 2.39	37.3* ± 2.26
Birth weight (gr)†	2615* ± 667	2162*± 544	3128* ± 325
Apgar (1. minute)†	8* ±1.44	6* ± 4.51	9* ± 0.37
Apgar (5. minute)†	9* ±0.76	6* ± 4.6	10* ± 0

*P = 0.001 differences between † Gr's 1 & 2 and 1 & 3 ; ↑ Gr's 1 & 2 and 2 & 3 ; † Gr's 1 & 2, 1 & 3 and 2 & 3

While there was not a single case in the control group, out of 70 women with PE, 19 (%27) women revealed IUGR fetuses, additional 2 (%2.85) had IUFD and 2 (%2.85) had PLABR. Group 2, on the other hand, consisted completely of IUGR, IUFD and PLABR but with no PE. Although both medical and / or surgical methods of induction of labor were applied more frequently in both groups 1 & 2 when compared with normal control cases (group 3) ($p = 0.018$ and $p = 0.001$, respectively). The most frequently induced group of all was the one with normotensive IPD as opposed to groups 1 & 3 ($p = 0.003$ & $p = 0.001$, respectively), with 40% rate. The mode of delivery was similar in all groups. Cesarean section rather than vaginal delivery was the preferred method in the whole study population regardless of whether they had complicated or normal pregnancies. **(Table 3)**

Table 3. Labor characteristics and mode of delivery in women with preeclampsia, other ischemic placental diseases and normal control groups (mean ± SD)

Parameter	Preeclamptic pregnancies (Gr I)		Other complications (Gr II)		Normal controls (Gr III)		
	n	%	n	%	n	%	p
Women	70		70		60		
Induction of Labor†	13	18.6	28	40	2	3.3	0.001*
Vaginal delivery	20	28.6	31	44.3	24	40	0.141
Cesarean delivery	50	71.4	39	55.7	36	60	0.141

* † difference between Gr's 1 & 2 (p = 0.003), 1 & 3 (p = 0.018) and 2 & 3 (p = 0.001)

Regarding the laboratory results, although Htc levels were similar in all groups. Group 2 revealed higher Hb levels than groups 1 & 3 (p = 0.013). PLT on the other hand, was lower only in women with PE (group 1) as opposed to groups 2 & 3 (p = 0.002). While serum Alb and CR levels were indifferent within the groups. UAC and Cu levels were higher in women with PE (group 1) (the highest of all) than groups 2 & 3 and also higher in group 2 than group 3 but lower than group 1 (p = 0.001 within all groups). Likewise vit B12 levels were significantly different between each group with the other (p = 0.001), the lowest being in group 1, then group 2 as compared to control cases. FOA, on the other hand, was lower in women with PE (group 1) (p = 0.001) in comparison to women with IPD (group 2) and the control group (group 3). The mean values of Htc, Alb and CR were similar in all groups. **(Table 4)**

Table 4. Laboratory parameters of women with preeclampsia, other ischemic placental diseases and normal control groups (mean ± SD)

Parameter	Preeclamptic pregnancies (Gr I)	Other complications (Gr II)	Normal controls (Gr III)	p
Women (n)	70	70	60	
Hemoglobin (gr/dl)†	11.79± 1.35	12.14±1.23*	11.48± 1.47	0.013*
Hematocrit (%)	34.99± 3.28	35.49±3.44	35.04± 3.10	0.842
Platelet ↑	198.4 ±61.2*	231.5 ±54.0	224.4 ±53.3	0.002*
Albumin (mg/dl)	3.36± 0.43	3.55±0.39	3.44± 0.19	0.292
Kreatinin (mg/dl)	0.68± 0.11	0.70±0.25	0.70± 0.09	0.181
Üric Asit (mg/dl)ŕ	3.79± 1.42*	3.23±1.02*	3.06± 0.38*	0.001*
Vit B12 (pg/ml)ŕ	223.8±95.1*	234.9 ±84.3*	276.8 ±69.8*	0.001*
Folic Asit (ng/ml)↑	10.53± 4.11*	13.48± 6.36	11.32±5.40*	0.002*
Cupper (µgr/dl)ŕ	210.9±56.5*	183.3 ±47.8*	121.2 ±25.0*	0.001*

* † difference between Gr 2 and Gr's 1 & 3 (p=0.013) ;↑ difference between Gr 1 and Gr's 2 & 3 (p=0.002) ; ŕ difference between each group with the other (p=0.001)

In terms of coagulation factors, while serum FBN, HCY, aPCR, PAI-1 were elevated and PS was decreased significantly (p = 0.001 in all differences) both in women with PE and IPD (Gr 1 & 2) when compared with the levels measured in the control group. PE group also revealed increased serum levels of PAI-1 than the IPD group (p = 0.019) **(Table 5).**

Table 5. Thrombophilia markers and serum homocysteine levels in women with preeclampsia, other ischemic placental diseases and normal control groups (mean ± SD)

Parameter	Preeclamptic pregnancies (Gr I)	Other complications (Gr II)	Normal controls (Gr III)	p
Women (n)	70	70	60	
FBN (mg/dL)†	422.2 ±85.5*	423.2 ±83.2*	327.4 ±66.6*	0.001 *
HCY (µmol/L)†	10.50± 3.6*	10.97± 3.26*	7.25± 2.44*	0.001*
Protein C (%)	101.6 ±18.3	103.2 ±20.3	109.7 ±16.8	0.825
Protein S (%)†	55.55±28.9*	53.64±27.35*	68.48±17.22*	0.001*
AT III (%)	87.14±21.46	89.75±19.59	90.15±13.08	0.740
PAI-1 (ng/dl)† ‡	84.06±32.35*	70.88±33.37*	30.53±12.14*	0.001*
aPCR (sec)†	121.4 ±33.2*	126.4 ±38.8*	99.2 ± 20.9*	0.001*

* † difference between Gr 1 and Gr's 2 & 3 (p=0.001) ;

‡ difference between Gr 1 and Gr's 2 (p=0.019)

Table 6 reveals the cut off levels of each parameter and the percentage of women with abnormal test result in the PE & IPD patient groups. As seen from the data, Cu in 85.7 & 68.6%, PAI-1 in 77.1 & 60%, aPCR in 31.4 & 37.1%, Hcy in 28.6 & 44.3%, respectively, were elevated and PS in 52.9 & 57.1% respectively was suppressed in PE & IPD groups. In **table 7**, relative risk ratios of developing PE & IPD with abnormal thrombophilia markers, Hcy and Cu blood levels are demonstrated. Odds ratios for each parameter were as follows; Cu OR 114 & 41.45; PAI-1 OR 64.12 & 28.50; PS OR 32.51& 38.66; aPCR OR 8.7 & 11.22; Hcy OR 7.6 & 15.10, respectively.

Table 6. The cut off levels and the percentage of women with abnormal test results in in women with preeclampsia, other ischemic placental diseases and normal control groups

Parameter		Preeclamptic pregnancies (Gr I)		Other complications (Gr II)		Normal controls (Gr III)		p
		n	%	n	%	n	%	
Women		70		70		60		
Protein S	<%48	37	52.9	40	57.1	2	3.3	0.001
(%)	>%48	33	47.1	30	42.9	58	94.7	0.001
PAI-1	<58.37	16	22.9	28	40.0	57	95.0	0.001
(ng/dl)	>58.37	54	77.1	42	60.0	3	5.0	0.001
aPCR	<138.48	48	68.6	44	62.9	57	95.0	0.001
(sec)	>138.48	22	31.4	26	37.1	3	5.0	0.001
HCY	<10.97	50	71.4	39	55.7	57	95.0	0.001
(µmol/L)	>10.97	20	28.6	31	44.3	3	5.0	0.001
Copper	<159.80	10	14.3	22	31.4	57	95.0	0.001
(µgr/dl)	>159.80	60	85.7	48	68.6	3	5.0	0.001

P = 0.001

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

	Preeclampsia		Ischemic Placental disease	
Parameter	Odds Ratio (OR)	Confidence interval (CI)	Odds Ratio (OR)	Confidence interval (CI)
PAI-1	64.12	17.6 - 232.5	28.50	8.12 - 100.34
Protein S	32.51	7.36 - 143.64	38.66	8.74 - 171.04
aPCR	8.7	2.4 - 30.88	11.22	3.19 - 39.50
HCY	7.6	2.1 - 27.1	15.10	4.31 - 52.87
Cupper	114	29.8 - 435.4	41.45	11.68 - 147.01

Conclusion

Recent studies suggest that although ischemic placental diseases like preeclampsia, fetal growth restriction, intrauterine fetal demise and other similar complications of pregnancy may differ in their clinical manifestations, they may be considered as one disease process that clinically manifests itself in an underlying continuum of mild disease to more severe disease states as pregnancy approaches term (Norwitz, 2006). Many studies also indicated that several thrombophilias have a high prevalence in women with ischemic placental diseases (Pabinger, 2005; Kujovich, 2004).

In our study, all of the pregnant women in both IPD and control groups were young and nulliparous with single fetuses, with no risk factors for and family history of PE (like personal history of chronic hypertension, diabetes mellitus, renal or any autoimmune diseases). Therefore, we studied a well-selected low risk group of pregnant women to minimize the confounding factors which might increase the risk of IPD's, namely PE, IUGR and IUFD to occur, other than thrombophilia. The mean ages of the pregnant women in all of our 3 groups were comparable. While older age, in general has been blamed for elevated risk of not only PE, but also of IUFD, preterm birth and IUGR regardless of parity (Lisonkova, 2010). There are also publications claiming that chronologic age per se may not be a good predictor of pregnancy outcome (Scholl, 1992). It has also been argued that current evidence on the association between maternal age and perinatal outcome remains largely clouded by age-related confounding factors (Ales, 1990). Therefore, it remains unclear how much maternal age itself contributes to poor pregnancy outcome rather than age-related co-morbidity. Our preeclamptic (Gr 1), but not the normotensive IUGR and IUFD patients (Gr 2) were overweight with an average BMI of above 25 (26.79 ± 4.42), when compared with the control group (24.83 ± 1.67, $p < 0.05$). High maternal BMI is associated with large number of pregnancy-related complications such as preeclampsia and eclampsia, presumably related to the presence of insulin resistance and associated endothelial disorder (Mbah, 2010). Besides the well-known risks of developing gestational hypertension and diabetes, obese women are also exposed to the risks of stillbirth and composite adverse pregnancy outcome (Crane, 2013). Smoking habit, which is considered to be a preventive measure for PE but hazardous for IUGR and IUFD was comparable in all groups (Voigt, 2013; Engel, 2013). The data indicate that human adverse reproductive and developmental effects are produced by caffeine also (Christian, 2001). Caffeine consumption increases Hcy levels, which is a risk factor for adverse perinatal outcomes, such as IUGR and PE, by acting as a vitamin B_6 antagonist (Shiraishi, 2013). Prevalence of PE was shown to be increase with daily consumption of tea (Wei, 2009) and also with more than four cups of coffee (Wergeland, 1997).

It has long been known that environmental influences, such as smoking, alcohol use, poor nutrition (Shu, 1995) and thrombophilic state (Franchi, 2004) leading to placental thrombosis have causative roles in impaired fetal growth (Crane, 2013).

Although the medical literature is full of reports suggesting that a significant percentage of IUGR and IUFD pregnancies exhibit thrombophilia (Kupferminc, 1999; Ofir, 2013) one can also encounter studies claiming just the opposite (Infante-Rivard, 2002).

In the present study, 19 out of 70 of our patients (27%) in the PE group (Gr 1) and 48 out of 70 patients (69%) in other IPD group (Gr 2) were diagnosed to carry growth restricted fetuses and therefore, had to be delivered early (34 weeks) whereas not a single case of IUGR was encountered in the control group. Considering that group 1 had 2 and group 2 had 22 cases of IUFD, one can imagine that at least some of these fetuses might have been growth restricted also during the earlier weeks of gestation. We recognize that thrombophilia; by causing intervillous thrombosis and consequent placental perfusion impairment could be the reason behind IUGR and also IUFD. This cascade may eventually result in PLABR. In our and other's series, PLABR was encountered only in thrombophilic cases but not in the normal control pregnant women groups (Ananth, 2010). The women in groups 1 & 2 in the present study, had shorter duration of pregnancy (34.2 & 35.4 wks, respectively), lower birth weight (2615 & 2162 gr, respectively) and apgar scores at 1 / 5 minutes (8/9 & 6/6, respectively) when compared to the control group (37.3 wks, 3128 gr and 9/10, respectively). Considering that delivery of the fetus is the only effective treatment of PE, IUGR, IUFD and PLABR, preterm delivery to save the life of the mother and the fetuses in IPD explains the shorter duration of pregnancy, lower birth weight and lower apgar scores.

An elevation in Hb or Htc is hypothesised to be the result of a combination of reduced plasma circulating volume and enhanced erythropoiesis because of underlying placental hypoxia (Troeger, 2006). A recent sudy even claimed that an Hb value below 11.0 g/dL would exclude the risk for severe PE to 100% (Soliman, 2012). In our series, although PE women had lower PLT counts and women with IUGR and IUFD had higher Hb levels than the other 2 groups. There was no case with anemia or thrombocytopenia in either one of the groups. Women with thrombocytopenia have been claimed to have higher rates of PE, HELLP syndrome, IUGR and IUFD, PLABR, labor induction, preterm deliveries and lower Apgar scores. Moreover, to reflect also the severity of the primary disease perhaps as a marker of perinatal complications (Parnas, 2006).The value of serum Alb in pregnancy-complicated hypertension is a significant indicator of disease severity and can be regarded as an important

marker of poor prognosis of perinatal outcomes (Seong, 2010). On the contrary, none of the biochemical markers are sufficiently accurate to recommend their use as an indicator or predictor of IUGR or IUFD in routine clinical practice (Conde-Agudelo, 2013; Hui, 2012).

Hyperuricaemia has been described commonly in PE pregnancies, often preceding the diagnosis of PE and historically was used as a diagnostic marker until proteinuria was preferred as a marker of maternal renal dysfunction. Fetuses exposed to hypoxia have been shown to have increased serum levels of purine metabolites which can cross into the maternal circulation to be degraded by maternal xanthine oxidase, explaining the relationship between hyperuricemia and fetal growth retardation (Kocijancic, 2013). In a recent study, one standard deviation increase in serum UAC level was associated with 2.3-fold increased odds of progression to PE and 1.5-fold increased odds of developing clinically significant adverse maternal or infant conditions irrespective of the progression to PE (Saugstad, 1975). Furthermore it has been claimed that hyperuricaemia in women with PE identifies a population prone to develop specifically maternal renal disease, small for gestational age (SGA) and preterm birth (Wu, 2012) and should be considered at least as important as proteinuria in predicting adverse outcome (Bellomo, 2011). The debate is still open whether UAC is a simple marker of disease or has a causal role in the development of PE and/or IUGR. Although serum UAC and CR are expected to be strongly interrelated, not all studies demonstrated both to be elevated (Homer, 2008). 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 have demonstrated that our cases with IPD (Gr 1 & 2) as compared to controls had normal and comparable serum Alb and CR but significantly higher UAC levels, supporting the view that hyperuricemia might be a component of the IPD and predictor of maternal and fetal complications.

There is considerable evidence concerning trace elements serum level changes associated with IPD's (Sarwar, 2013), possibly due to the oxidative stress following deprived placental perfusion and ischemia (Kontic-Vucinic, 2006). Deficiency of Cu, which is an essential co-factor for the enzymes catalase, superoxide dismutase and cytochrome oxidase, can lead to a variety of vascular and nutritional disorders (Alebic-Juretic, 2005). There are some published studies that show statistically significant higher levels of Cu in the circulation of women with PE in comparison to healthy pregnant women (Alebic-Juretic, 2005; Serdar, 2006; Kumru, 2003). In others though, Cu serum levels are significantly higher both in healthy and pathological pregnancies (Katz, 2012; Frenzl, 2013) or even lower maternal serum concentrations of Cu have been described (Farzin, 2012). In another study,

IUGR cases showed higher magnesium, copper, and selenium concentrations in umbilical cord arterial sera and higher magnesium and selenium concentrations in placental tissue, but no significant differences appeared for the elements measured in maternal and umbilical cord venous sera (Osada, 2002).

In the present study, the serum Cu levels were significantly elevated both in groups 1 & 2 versus normal pregnant women, so much so that more than 86% of preeclamptic women and 69% of women with IUGR and IUFD revealed levels above the normal treshold. As a matter of fact, the likelihood ratio of developing PE or IUGR and IUFD in women with elevated Cu levels was 114 and 41, respectively. Although this finding is very stimulating, even intriguing, similar data have to be reproduced by others.

Hyperhomocysteinemia is reported in women with PE and / or IUGR versus age-matched normotensive pregnant control subjects (Hogg, 2000). The levels of vit B12 and FOA were reported to be low in most (Acilmis, 2011; Lachmeijer, 2001), as yet unchanged in in others as compared to control groups (Hogg, 2000; Gadhok, 2011). Recent studies revealed that increasing serum Hcy levels in pregnancies complicated with IUGR were accompanied by decreasing levels of serum FOA and vit B12 (Furness, 2013). In the present study, both preeclamptic and IUGR patients also revealed higher Hcy and lower FOA and B12 levels in accord with the previous studies. In a review, the presence of high Hcy values increased the relative risk of IPD 1.32–3.2, while the RR reached 9.7 in primiparas and 6.9 in obese patients (Powers, 1998). In our series, in primiparas, the likelihoods of development of PE and IUGR were found to be 7 times and 15 times higher, respectively in women with elevated Hcy serum levels. Hyperhomocysteinemia was encountered in %28 of women with PE and in 44% of women with IUGR, in the present study.

Hyperfibrinogenaemia is associated with systemic arterial and venous thromboembolism and therefore may contribute to placental vascular disease associated with obstetric complications (Pabinger, 2005; Kujovich, 2004). Fibrinogen was found to be significantly elevated above controls in our groups of PE and IUGR.

The primary aim of this study was to explore the magnitute of the impact of inherited thrombophilia on IPD's among a pregnant group of Turkish women. In this study, since mutations in the factor V mutations (FVL) are related to activated protein C resistance (aCPR) and FVL is reported in about 90% of patients with aPCR in the general population (Bernardi, 1997) we measured aPCR as a marker.

We have clearly demonstrated that FBN, Hcy, PAI-1 and aPCR values are higher and PS values are lower in women with PE, IUGR and IUFD, when compared to our normotensive normal pregnant control group

(Table 5). The percentages of women with abnormal test results in women with PE and IUGR & IUFD are 77 and 60% for PAI-1, 47 and 43% for protein S, 31 and 37% for aPCR, 29 and 44% for Hcy, respectively (Table 6). Regarding the likelihood ratios of developing PE and IUGR & IUFD of women with abnormal blood levels of thrombophilia markers are [OR 64 and 28] for PAI-1; [OR 32 and 38] for PS; [OR 9 and 11] for aPCR and [OR 7 and 15] for Hcy, respectively (Table 7). Among all the laboratory parameters that we studied in our research, serum Cu levels predicted both the PE and IUGR & IUFD, the most with an odds ratio of 114 and 41, respectively, which was found to be elevated in more than 85% of preeclamptic and 68% of women with IUGR & IUFD. In contrast to some previous studies (Robertson, 2006), we did not find an increased frequency of supressed PC and AT III levels in women with PE as compared to control women.

Several recent metaanalyses that have attempted to analyze the collective data have reported that pooled OR for associations between inherited thrombophilia and IPD are generally in the 1.0 to 2.5 range, indicating that thrombophilia may be only weakly associated with PE, if at all (Lin, 2005). Based on the results of this present study of ours, we claim that the association between thrombophilia and PE & IUGR & IUFD is stronger than suggested by the majority of publications in the medical literature. The most important of all, other than thrombophilia, Cu as an independant marker is selectively and remarkably elevated in women with complicated pregnancies 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 IPD's exist, Cu could be a valuable tool to predict complications of pregnancy.

The current case control study presents some limitations such as the study being done in a tertiary center, thereby not reflecting the general Turkish population and the small number of patients enrolled. Furthermore, prothrombin gene (G20210A) mutation was not tested in this study. One of the strengths is that potential confounding factors such as age, ethnicity, and systemic medical problems are 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 IPD is causal or temporal, more specifically whether thrombophilia acts as a cofactor in the pathogenesis of these diseases or accelerates their course is, speculative. Lastly, even the strong association detected between PE, IUGR and IUFD and inherited thrombophilia in this study should not be considered as a

justification for screening for thrombophilia since there is no clear evidence that anticoagulation can prevent or improve the outcome.

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