Effects of inherited trombophilia in women with recurrent pregnancy loss

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Summary

Purpose of Investigation: To evaluate the prevalence and effects of inherited thrombophilia caused by factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase (MTHFR) C677T mutations in women with recurrent pregnancy loss. *Methods:* A study group of 97 women with recurrent miscarriages and a control group of 71 healthy pregnant women were included in the study. Genotype analyses for factor V Leiden, prothrombin G20210A and MTHFR C677T polymorphisms were performed by real-time polymerase chain reaction (RT-PCR). *Results:* The frequency of factor V Leiden, prothrombin G20210A and MTHFR C677T mutations were similar in both the study and control group. There were eight patients (8.2%) who had more than one gene mutation in the study group and one patient in the control group (1.4%). This difference was not statistically significant. Study group patients (n = 97) were compared in terms of the number of miscarriages and the abortion week, in addition to being a carrier of factor V Leiden and MTHFR C677T gene mutations. No statistically significant correlation was found between being a factor V Leiden and MTHFR C677T mutation carrier with either the number of miscarriages or the abortion week. *Conclusion:* Factor V Leiden, prothrombin G20210A and MTHFR C677T gene mutations are not individually related with recurrent pregnancy loss. However, combined gene mutation status may be associated with recurrent miscarriages.

Key words: Recurrent pregnancy loss; Factor V Leiden; Prothrombin; MTHFR.

Introduction

Recurrent pregnancy loss (RPL) is traditionally defined as three or more consecutive miscarriages before the 20th week of pregnancy. According to this definition, RPL occurs in one of 100 pregnancies [1]. This frequency increases to 5% when it is defined as two or more pregnancy losses [2, 3]. Parental chromosomal anomalies, uterine anomalies, endocrine disorders, infections, immunological factors, acquired or inherited thrombophilia, and maternal chronic diseases contribute to approximately 50% of cases. However, in the remaining 50% the etiology is unknown [4].

As pregnancy is a hypercoagulable state, secondary to an increase in coagulation factors and a reduction in naturally occurring anticoagulants, thrombophilia may exaggerate this hypercoagulability and diminish uteroplacental circulation. For this reason, maternal thrombophilias (factor V Leiden, prothrombin, methylenetetrahydrofolate reductase (MTHFR) C677T mutations, and deficiencies of protein C and protein S) may be related to adverse pregnancy outcomes and significant pathologies in obstetrics [5]. When the prevalence of gene mutations for factor V Leiden, prothrombin and MTHFR were compared among women with RPL, and without any miscarriage, results were conflicting [6-9].

The aim of this study was to identify the possible role of factor V Leiden, prothrombin G20210A and MTHFR C677T gene mutations in patients with RPL in the Western region of Turkey.

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Materials and Methods

In this study we examined 97 patients with RPL and 77 patients without RPL in the period between January 2006 and May 2009. The criterion to accept patients as cases of RPL was two or more miscarriages before the 20th week of gestation. Women with anatomic, autoimmune, hormonal, or chromosomal abnormalities, or antiphospholipid antibody syndrome were excluded.

The control group consisted of 71 patients (18-37 years old) who had given birth at least once without any problems during pregnancy, who had a maximum of one abortion in their history, and were without any personal or familial history of thromboembolism.

Before inclusion in the study, all the patients underwent physical examination, blood pressure measurement, and hematological and urinary examination to exclude chronic hypertension, chronic nephropathy and other major systemic diseases. None of the women who had previous thromboembolic diseases or family history were included in the study.

All patients, whether in the study or control group, were examined for factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations. For DNA isolation, peripheral blood was collected in EDTA tubes with the consent of all participating women. Genotype analysis was performed by real-time (online) polymerase chain reaction (RT-PCR).

Factor V Leiden (G1691A) and prothrombin (G20210A) mutations

Genomic DNA was extracted from peripheral leukocytes of the subjects using the High Pure PCR Template Preparation Kit (Roche Applied Science; Mannheim, Germany). All patients were tested for the presence of factor V Leiden and prothrombin G20210A mutations on the Lightcycler™ system using the commercial LightCycler Factor V Leiden G1691A and Prothrombin G20210A Mutation Detection Kits, respectively (Roche Diagnostics; Mannheim, Germany).

Genotyping of the different alleles for the factor V Leiden mutation was done according to the specific melting temperature (Tm) of the resulting amplicons. In this analysis a wildtype genotype with two copies of the G allele (G/G) showed a single melting peak at 65°C, a mutant genotype with two copies of the A allele (A/A) also showed a single melting peak but at 57°C, and a heterozygous genotype with both alleles (G/A) showed two melting peaks at 65°C and 57°C.

Similarly, specific Tm of the resulting amplicons identified different alleles of the prothrombin G20210A mutation. In this analysis a wildtype genotype with two copies of the G allele (G/G) showed a single melting peak at 59°C, a mutant genotype with two copies of the A allele (A/A) also showed a single melting peak but at 49°C, and a heterozygous genotype with both alleles (G/A) showed two melting peaks at 59°C and 49°C.

MTHFR (C677T) mutation

For the detection of the C677T polymorphism at the MTHFR gene, specific primer probes were used together with the Light-Cycler-DNA Master Hybridization Probes Kit (Roche Applied Science; Mannheim, Germany). Experiments were carried out on the LightCycler™ system (Roche Applied Science; Mannheim, Germany) according to the protocol of Charalampos Aslandis and Gerd Schmitz (Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Regensburg, Germany). Specific Tm of the resulting amplicons identified polymorphic alleles. In this analysis individuals with two copies of the C allele (C/C) showed a single melting peak at 63.1°C, individuals with two copies of the T allele (T/T) also showed a single melting peak but at 54.6°C, and individuals with both alleles (C/T) showed two melting peaks at 54.6°C and 63.1°C.

Statistical analysis

All statistical analyses were performed using Microsoft SPSS 11.0 version. Continuous variables were assessed using the Student's t-test. A chi-square test was applied to detect the statistical differences between genetic mutations in the RPL group and control group. Data are presented as the mean ± SD. Differences were considered to be significant at $p < 0.05$.

Results

The mean age of the study group (n = 97) was 30.6 ± 5.8 (20-43 years old) and that of the control group (n = 71) was 28.2 ± 5.2 (19-38 years old). There was a statistically significant difference between the mean ages of the two groups ($p < 0.05$). The number of abortions in the study group (n = 97) was 2.9 ± 1.2 (2-7) on average. The mean abortion week of the study group was 8.4 ± 2.9 (weeks 4-19), whereas it was 7.1 ± 1 (weeks 6-9) in the control group. Twenty-nine patients had miscarriages in the second trimester. However, it was found that 26 of these patients also had at least one first-trimester loss. There was no statistically significant difference between the two groups in terms of abortion week .

In terms of the factor V Leiden gene, within the study group (n = 97) 86 patients (88.7%) had normal genotypes (GG), nine (9.2%) had heterozygote (GA) genotypes, and two patients (2.1%) had homozygote (AA) genotypes. In the control group (n = 71) 64 patients (90.1%) had normal genotypes, six patients (8.5%) had heterozygote genotypes, and one patient (1.4%) had a homozygote genotype. There was no statistically significant difference between the two groups in terms of being a carrier of the factor V Leiden mutation (Table 1).

Table 1. — *Factor V Leiden and prothrombin G20210A gene mutation status in both groups.*

	Factor V Leiden				Prothrombin G20210A			
	Normal genotype	Heterozygote genotype	'A' allele carrier	*p* value	Normal genotype	Heterozygote genotype	'A' allele carrier	*p* value
Study group (n = 97)	86 (88.7%)	9 (9.2%)	2 (2.1%)	n.s.	94 (96.9%)	3 (3.1%)	3 (3.1%)	n.s.
Control group (n = 71)	64 (90.1%)	6 (8.5%)	1 (1.4%)		70 (98.6%)	1 (1.4%)	1 (1.4%)	

n.s. = non significant.

Table 2. — *MTHFR C677T gene mutation status in both groups.*

	MTHFR C677T			
	Normal (CC) genotype	Heterozygote (CT) genotype	Homozygote (TT) genotype	T allele carrier
Study group (n = 97)	46 (47.4%)	31 (32%)	20 (20.6%)	51 (52.6%)
Control group (n = 71)	34 (47.9%)	30 (42.3%)	7 (9.9%)	37 (52.1%)
p value	n.s.	n.s.	n.s.	n.s.

n.s. = non significant.

Table 3. — *Combined gene mutation status in both groups.*

Combined gene mutation		
Study group (n = 97)		Control group (n = 71)
8 (8.2%)	n.s.	1 (1.4%)

n.s. = non significant.

In terms of the prothrombin G20210A gene mutation, gene mutation carrier status was identified in three patients (3.1%) in the study group (n = 97), whereas a heterozygote gene mutation was found in one patient (1.4%) in the control group (n = 71). No statistically significant difference was found between the two groups in terms of carrier status for the prothrombin G20210A gene mutation (Table 1).

In terms of the MTHFR C677T gene mutation, in the study group (n = 97) 46 patients (47.4%) had normal (CC) genotypes, 31 patients had (32%) heterozygote (CT) genotypes, and 20 patients (20.6%) had homozygote (TT) genotypes. In the control group (n = 71) 34 patients (47.9%) had normal genotypes, 30 patients (42.3%) had heterozygote genotypes, and seven patients (9.9%) had homozygote genotypes. There was no statistically significant difference between the groups in terms of MTHFR C677T gene mutation carrier status (Table 2).

It was found that eight patients (8.2%) in the study group (n = 97), and one patient (1.4%) in the control group were carriers of multiple gene mutations. This difference was not statistically significant (Table 3).

Study group patients (n = 97) were compared in terms of the number of miscarriages and the abortion week, as well as carrier status of the factor V Leiden mutation. In

Table 4. — *Number of abortions and abortion weeks for factor V Leiden 'A' allele carriers and MTHFR C677T 'T' allele carriers within study group.*

	Factor V Leiden			Prothrombin G20210A		
	A allele carriers (n = 11)	Non carriers (n = 86)	*p* value	T allele carriers (n = 51)	Non carriers (n = 46)	*p* value
Number of miscarriages (mean ±SD)	3.3 ± 1.5	2.8 ± 1.2	n.s.	2.9 ± 1.1	2.9 ± 1.3	n.s.
Abortion week (mean ± SD)	8.6 ± 2.5	8.5 ± 2.3	n.s.	8.7 ± 2.4	8.4 ± 2.2	n.s.

n.s. = non significant.

the 86 non-carrier patients the average number of miscarriages was 2.8 ± 1.2 and the mean abortion week was 8.5 ± 2.3, while the average number of miscarriages for the 11 patients with 'A' allele carrier status was 3.3 ± 1.5 and the mean abortion week was 8.6 ± 2.5. Accordingly, there was no statistically significant correlation between being a carrier of the factor V Leiden mutation with either the number of miscarriages or the abortion week (Table 4).

Study group patients (n = 97) were compared in terms of the number of miscarriages and the abortion week, as well as being a carrier of the MTHFR C677T gene mutation. In the 46 non-carrier patients the average number of abortions was 2.9 ± 1.3 and the mean abortion week was 8.4 ± 2.2, while the average number of abortions for the 51 patients with T allele carrier status was 2.9 ± 1.1 and the mean abortion week was 8.7 ± 2.4. Accordingly, no statistically significant correlation was found between being a MTHFR C677T mutation carrier with either the number of miscarriages or the abortion week (Table 4).

Discussion

Factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations, which are known to increase the risk of thrombosis, are thought to play a role in RPL and in the etiology of late pregnancy complications [10, 11]. In the literature, there are case control studies investigating the relationship between factor V Leiden mutation and RPL that are similar to our study in terms of study design and patient selection. Although some of these studies have shown a link between factor V Leiden mutation and RPL [12, 13], a significant number of studies has also failed to identify such a relationship [8, 14].

In a meta-analyses, the factor V Leiden mutation was found to be associated with RPL and these women were found to be at higher risk of pregnancy loss in the second compared with the first trimester (OR 7.83, 95% CI 2.83-21.67 and OR 2.01, 95% CI 1.13-3.58, respectively) [15]. This finding was supported by another meta-analysis by Robertson *et al.* [16] (OR 4.12; 95% CI 1.93-8.81 and OR 1.91; 95% CI 1.01-3.61, respectively). However, in a study by Rai *et al.* [17], which comprises one of the largest patient populations among the studies conducted to this point, 905 unselected patients with RPL and 150 control cases were examined and it was concluded that there was no relationship between the factor V Leiden mutation and RPL. In our study, carrier status of factor V Leiden mutation was found to be 11.3% in the study group and 9.9% in the control group and these rates were not considered statistically significant.

As is the case with factor V Leiden, there are numerous studies that have investigated the effects of the prothrombin G20210A mutation on RPL and found that prothrombin G20210A heterozygosity increases the risk of RPL from 2-6.5 times [15, 16]. On the other hand, Brenner *et al.* [18], Wramsby *et al.* [19], and Kutteh *et al.* [20] conducted three case-control studies examining the relationship between the prothrombin G20210A mutation and RPL, and reported that the mutation did not increase the risk of pregnancy loss. In our study, although the difference was not statistically significant, carrier status of the prothrombin G20210A mutation was found to be 3.1% in the study group whereas it was 1.4% in the control group.

The results of studies on the effects of the MTHFR C677T gene mutation on unexplained RPL remain contradictory. These conflicting results may be attributed to the number of patients included in the studies, and the variability of selection criteria for study and control group cases, as well as differences in ethnic and geographical distribution of the T allele. The majority of researchers agree that the MTHFR C677T heterozygote mutation does not lead to pregnancy loss. However, Martinelli *et al.* [21] and Nelen *et al.* [22] reported that the MTHFR C677T homozygote mutation leads to mild hyperhomocysteinaemia, and that it may increase the risk of pregnancy loss by 2-3 times. In a study by Jivraj *et al.* [23], 714 women were selected for the case group and 136 women were selected for the control group. The rate of carrier status of the MTHFR C677T mutation was the same (32%) in both groups. While achieving these results, Jivraj *et al.* [23] did not evaluate the heterozygote and homozygote MTHFR C677T mutation separately. In a meta-analysis (26 case-controlled studies, 2120 RPL cases and 2949 controls), Ren *et al.* [24] stated that women with RPL have three times more homozygote MTHFR C677T mutations and that this is significant with regards to RPL. However Rey *et al.* [15] could not find any relation between the MTHFR homozygote mutation and RPL. Meta-analyses by Robertson *et al.* [16] and Nelen *et al.* [25] also showed that hyperhomocysteinaemia was strongly associated with RPL (OR 6.25; 95% CI, 1.37-28.42 and OR 4.2; 95% CI, 2.0-8.8 respectively). According to all these studies, it could be concluded that hyperhomocysteinaemia plays the major role in RPL instead of MTHFR gene mutation.

In a study investigating the relationship between RPL and combined thrombophilic mutations, conducted by Sotiriadis *et al.* [26], 88 patients with RPL were compared to 88 controls. The relationship between combined thrombophilic mutations and RPL was found to be statistically nonsignificant. Our study supports Sotiriadis *et al.*, as we have found combined gene mutations are not

statistically significant in terms of RPL, however, the frequency was higher (8.2% vs 1.4%). In a study by Coulam *et al.* [27], 150 women with a history of RPL and 20 fertile control were examined for ten thrombophilic gene mutations. They concluded that while none of the specific thrombophilic gene mutations appears to be a risk factor for RPL, when taken together the total number of mutations is a significant risk.

Conclusion

Although the number of cases is not adequate to make definitive comments, our results suggest that factor V Leiden, prothrombin G20210A and MTHFR C677T gene mutations are not individually related with RPL. However, combined gene mutation status may be associated with RPL as the frequency was higher (8.2% vs 1.4%). Further studies with larger series are needed to clarify this issue.

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