The association between inherited thrombophilia and recurrent pregnancy loss in Turkish women

U. Isaoglu[1], P. Ulug[2], I.B. Delibas[1], M. Yilmaz[3], Y. Kumtepe[3], H. Dogan[4], S. Tasdemir[5]

[1]Nenehatun Obstetrics and Gynecology Hospital, Erzurum
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Erzincan University, Erzincan
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Ataturk University, Erzurum
[4]Department of Medical Biology, Faculty of Medicine, Ataturk University, Erzurum
[5]Department of Medical Genetic, Faculty of Medicine, Ataturk University, Erzurum (Turkey)

Summary

Objective: To investigate the relation between recurrent pregnancy loss (RPL) and factor V Leiden, prothrombin G20210A, and C677T methylenetetrahydrofolate reductase (MTHFR) mutations. *Materials and Methods:* A case-control study was conducted on 95 consecutive cases with RPL, and 40 age-matched controls who had no history of pregnancy loss and had at least one successful pregnancy. After application of exclusion criteria, 60 patients in the study group and 40 control cases were compared for thrombophilic factors. *Results:* Thirteen out of 60 RPL cases and one out of 40 in the control group were carriers of factor V Leiden mutation. While six patients were carriers of prothrombin G20210A gene mutation, none in the control group carried this mutation. Twenty-nine out of 60 RPL cases and 17 out of 40 control cases had MTHFR mutation. *Conclusion:* The authors found a positive correlation between RPL and FVL and FII gene mutations, but no significant association between RPL and MTHFR gene mutation.

Key words: Recurrent pregnancy loss; Factor V Leiden; Prothrombin G20210A mutation; MTHFR C677T mutation.

Introduction

Recurrent pregnancy loss (RPL) is a frequent health problem, with three or more losses affecting one to two percent and two or more losses affecting up to five percent of women in the reproductive age [1, 2]. While several etiologies have been implicated to play a role in RPL including chromosal translocations and inversions, anatomic alterations of the uterus, endocrinologic abnormalities, and autoimmune disorders [3, 4], until recently the majority of RPL remained unexplained. Association with acquired thrombophilia, such as antiphospholipid antibodies and RPL, is well established. Based on the histological findings of extensive infarction and necrosis in the placentas of women with antiphospholipid syndrome, researchers postulate that uteroplacental thrombosis may lead to placental infarction and eventual fetal death [5]. A number of studies in women with inherited thrombophilia have also suggested an association with fetal loss.

The three most common genetic thrombophilias known to predispose to venous thrombosis are: factor V Leiden (FVL), methylenetetrahydrofolate reductase mutation (MTHFR, C677T) [6, 7], and prothrombin gene mutation (FII, G20210) [8]. FVL mutation involves a G→A substitution at nucleotide 1691 of coagulation factor V gene [9]. Factor Va becomes resistant to degradation by activated protein C due to this substitution. This mutation in the factor V gene increases the risk of venous thromboembolism three- to five-fold in heterozygous individuals [10]. One genetic variation, a G to A transition at nucleotide position 20210, in the 3'-untranslated region of the coagulation factor II gene, has been found to be associated with increased prothrombin levels and risk for venous thrombosis [8]. This mutation is quite common in the normal population (0.7% - 4.0%) [11], whereas it is responsible for 6.2% [8] of all the cases of thromboses. MTHFR deficiency is the most common congenital error of folate metabolism, which leads to elevated homocysteine plasma levels. A common mutation in the MTHFR gene, i.e. a cytosine to thymine transition at position 677, is associated hyperhomocysteinemia which predisposes to thrombosis [12, 13].

The aim of this study was to evaluate the prevalence of FVL, prothrombin G20210A, and C677T MTHFR mutations in women with recurrent fetal loss in the Turkish population.

Materials and Methods

This study was performed in Ataturk University Faculty of Medicine, Department of Obstetrics and Gynecology, between January 2007 and March 2008. In this case-control study the prevalence of factor V Leiden, prothrombin G20210A and C677T MTHFR mutations were determined in a consecutive series of 95 women referred for evaluation of recurrent spontaneous pregnancy loss (study group patients) and 40 women with at least one successful pregnancy and no history of pregnancy loss (controls).

The patients with recurrent pregnancy loss were Turkish women (age range 19-46 years; mean 29.14 ± 6.18), referred for evaluation at a university hospital. The clinical details of each patient and her pregnancy losses were recorded, paying particular attention to whether the previous pregnancy losses occurred in the early pregnancy period (first trimester, ≤ 12 weeks of gestation) or late pregnancy period (> 12 weeks of gestation), and whether

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Table 1. — *Charactarictics of RPL patients with additional pathology.*

	RPL group with additional pathology (n = 35)	FII G20210A carriers (n = 2)	FVL mutation carriers (n = 2)	MTHFR mutation carriers (n = 15)
Age (years; mean)	30.1 ± 6.3	33.5 ± 7.8	27.5 ± 3.5	30.9 ± 6.3
Defined causes				
Anatomical	7	1	2	0
Hormonal	5	0	0	1
Chromosomal	2	1	0	1
Autoimmune	13	0	0	2
Other coagulation disorders*	3	0	0	3
Different combination of these causes	5	0	0	8
Total	35	2	2	15

*Deficiencies of antithrombin III, protein C and protein S.

Table 2. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between the RPL patients and the controls.*

Type of genetic defect	Recurrent pregnancy loss (n = 60)	Controls (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	13 (21.7)	1 (2.5)	10.8 (1.35-86.16)	0.007
Prothrombin G20210A mutation n (%)	6 (10)	0 (0)	Not calculated	0.039
Either mutation n (%)	19 (31.7)	1 (2.5)	18.07 (2.31-141.53)	<0.001

the patients were primary or secondary RPL (primary RPL are women with no previous live births, secondary if there was a live birth followed by pregnancy losses. Eligibility criteria for the study group was a history of two or more spontaneous pregnancy losses. Forty-six women had two pregnancy losses, 34 had three, and 15 had more than three. Sixty-eight out of 95 patients had only early, five had early and late, and 22 had late pregnancy losses. All women had been previously investigated for autoantibodies, glucose tolerance test, HbA1C levels, thyroid function, serum prolactin levels, coagulation disorders other than factor V Leiden, MTHFR and prothrombin G20210A polimorphism, uterine anatomic anomalies with hysterosalpingography (HSG,) and karyotype of both parents. Of these women; two had abnormal karyotype, seven had uterine septum, four were positive for antiphospholipid antibodies (APA), nine had autoantibodies other than APA, 13 had different combination of other pathologies, such as diabetes mellitus, thyroid dysfunction, hyperprolactinemia, and deficiencies of antithrombin III, protein C, and protein S (Table 1). None of the patients had a history of thrombo-embolic event.

The control group consisted of 40 age-matched women (age range 19-45 years, mean 30.50 ± 6.77) with no previous pregnancy loss and thrombo-embolic events. Both study patients and control subjects were born in the east of Turkey and were living in Erzurum province or a nearby region.

Total genomic DNA was isolated from peripheral vein blood samples with a MagNA Pure LC DNA Isolation Kit using a MagNA Pure LC 2.0 Automated DNA isolation instrument . The FVL, Factor II, and MTHFR kit allowed mutation genotyping using a Lightcycler 2.0 Instrument.

Data were stored and analysed using SPSS (Statistical Package for Social Science, release 15.0) in an IBM-compatible computer. The chi-square and Student's t test were used to assess intergroup significance. In addition, the odds ratios (OR) and 95% CI were estimated separately for each polymorphism. The difference was considered as statistically significant when $p < 0.05$.

Results

Thirty-five patients out of 95 were not included in the statistical analysis because they had additional pathology and remaining 60 were included in order to investigate the relationship between the RPL and FVL, prothrombin G20210A and C677T MTHFR mutations. Mean age of the RPL group without additional pathology and the control group was 28.57 ± 6.1 and 30.50 ± 6.8 years, respectively ($p > 0.05$). The 60 patients in the study group had 177 previous pregnancy losses (mean: 2.95 ± 1.65). Forty patients out of 60 (66.7%) were diagnosed as having at least one thrombophilia marker, whereas 20 (33.3%) had no thrombophilia.

Concerning the FVL mutation, 13 out of 60 RPL patients and one out of 40 controls carried FVL mutation (21.7 vs. 2.5%, $p = 0.007$, odds ratio 10.8, 95% CI: 1.35 - 86.16). No factor V Leiden homozygosity was found in the RPL and control groups (Table 2). Forty-six out of 60 RPL patients had early and 14 had late pregnancy losses. 11 out of 46 patients with early pregnancy loss, and one out of 40 controls carried FVL mutation (23.9 vs 2.5%, $p = 0.004$, odds ratio 12.26, 95% CI: 1.51 - 99.83). Two out of 14 patients with late pregnancy loss and one out of 40 controls carried FVL mutation (14.3 vs 2.5%, $p = 0.09$, odds ratio 6.5, 95% CI: 0.5 - 78.1). The prevalence of FVL mutation was higher in the group of late pregnancy loss, but the difference did not reach statistical significance (Table 3). Of the entire study group of 60 women, 41 were primary RPL, whereas 19 were secondary RPL. Twelve out of 41 patients who had primary RPL and one out of 40 controls carried the FVL mutation (29.3 vs 2.5%, $p = 0.001$, odds ratio 16.14, 95% CI: 1.98 - 131.24). One out of 19 patients who had secondary RPL and one out of 40 controls carried the FVL mutation (5.3 vs 2.5%, $p = 0.3$, odds ratio 2.17, 95% CI: 0.13 - 36.62). The prevalence of FVL mutation was higher in the group of secondary RPL, but the difference did not reach statistical significance (Table 4). Thirteen out of 60 RPL patients without additional pathology and two out of 35 patients with additional pathology carried the FVL mutation (21.7 vs 5.7 %, $p = 0.040$, odds ratio 4.56, 95% CI: 0.97 - 21.59) (Table 5).

Concerning the prothrombin G20210A polymorphism, six prothrombin G20210A mutations were observed in the RPL group, whereas none of the controls had prothrombin G20210A mutation (10% vs 0%, $p = 0.039$, odds ratio was not calculated since none of the controls had prothrombin G20210A mutation) (Table 2). No prothrombin (FII)

Table 3. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with early and late RPL patients and the controls.*

Type of genetic defect	Early RPL (n = 46)	Controls (n = 40)	Odds ratio (95% CI)	*p* value	Late RPL (n = 14)	Controls (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	11 (23.9)	1 (2.5)	12.26 (1.51-99.83)	0.004	2 (14.3)	1 (2.5)	6.5 (0.5-78.1)	0.09
Prothrombin G20210A mutation n (%)	5 (10.87)	0 (0)	Not calculated	0.032	1 (7.14)	0 (0)	Not calculated	0.089
Either mutation n (%)	16 (34.8)	1 (2.5)	20.8 (2.6-165.8)	< 0.001	3 (21.4)	1 (2.5)	10.6 (1.0-112.7)	0.02

Table 4. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with primary and secondary RPL patients and controls.*

Type of genetic defect	Primary RPL (n = 41)	Controls (n = 40)	Odds ratio (95% CI)	*p* value	Secondary RPL (n = 19)	Controls (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	12 (29.3)	1 (2.5)	16.14 (1.98-131.2)	0.001	1 (5.3)	1 (2.5)	2.17 (0.13-36.62)	0.3
Prothrombin G20210A mutation n (%)	5 (12.2)	0 (0)	Not calculated	0.023	1 (5.26)	0 (0)	Not calculated	0.14
Either mutation n (%)	17 (41.5)	1 (2.5)	27.6 (3.45-221.1)	< 0.001	2 (10.5)	1 (2.5)	4.59 (0.39-54.09)	0.19

Table 5. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with and without additional pathology.*

Type of genetic defect	RPL patients without additional pathology (n = 60)	RPL patients with additional pathology (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	13 (21.7)	2 (5.7)	4.56 (0.97-21.59)	0.040
Prothrombin G20210A mutation n (%)	6 (10)	2 (5.7)	1.83 (0.35-9.62)	0.47
Either mutation n (%)	19 (31.7)	4 (11.4)	3.59 0.026 (1.11-11.63)	

Table 6. — *Comparison of the prevalence of C677T methylenetetrahydrofolate reductase mutation between RPL patients and controls.*

Type of genetic defect	RPL patients (n = 60)	RPL patients (n = 40)	Odds ratio (95% CI)	*p* value
C677T methylenetetrahydrofolate reductase mutation n (%)	29 (48.3)	17 (42.5)	1.27 (0.56-2.83)	0.57
Homozygous n (%)	1 (1.67)	3 (7.5)	0.21 (0.02-2.09)	0.15
Heterozygous n (%)	28 (46.67)	14 (35)	1.63 (0.71-3.71)	0.25

G20210A homozygosity was found in the RPL group. Five out of 46 patients with early pregnancy loss (10.87% vs 0%, $p = 0.032$) and one out of 14 patients with late pregnancy loss (7.14% vs 0%, $p > 0.05$) carried the FII G20210A mutation, whereas none of the controls had prothrombin G20210A mutation. The prevalence of FII G20210A mutation was higher in the group of late pregnancy loss, but the differences did not reach statistical significance (Table 3). Five out of 41 patients who had primary RPL (12.2% vs 0%, $p = 0.023$) and one out of 19 patients who had secondary RPL (5.26% vs 0%, $p > 0.05$) carried the FII G20210A mutation, whereas none of the controls had prothrombin G20210A mutation (Table 4). Six out of 60 RPL patients without additional pathology and two out of 35 patients with additional pathology carried the FII G20210A mutation (10% vs 5.7%, $p > 0.05$) (Table 5).

Concerning the C677T MTHFR mutation, 29 out of 60 RPL patients and 17 out of 40 controls had C677T MTHFR mutation (48.3% vs 42.5%, $p = 0.566$, odds ratio:1.27, 95% CI: 0.56 - 2.83). Among the RPL patients with C677T MTHFR mutation, only one patient was homozygote and the rest of the patients (n = 28) were heterozygote, whereas three patients out of 17 controls with C677T MTHFR mutation were homozygote and the remaining 14 were heterozygote (Table 6).

Two women with RPL were compound heterozygote, i.e. carrier of both the FII G20210A and C677T MTHFR mutation, whereas six RPL women were compound heterozygote, i.e. carrier of both the FVL and C677T MTHFR mutation.

These results suggest that factor V Leiden and prothrombin G20210A mutation, but not C677T MTHFR mutation, may be predisposing factors for RPL and that the prevalence of both FVL and prothrombin G20210A mutation are more prominent in early and primary RPL patients.

In order to investigate whether women with three or more RPL more frequently carry the FVL and prothrombin G20210A mutations than women with only two RPL, the prevalence of these two mutations is compared between the RPL patients and the controls. Five out of 28 women with two RPL (17.9%) and eight out of 32 with three or more

(25%) carried the FVL mutation ($p = 0.50$). Three out of 28 women with two RPL (10.7%) and three out of 32 with three or more (9.4%) carried the prothrombin G20210A mutation ($p = 0.86$).

Discussion

This study revealed a strong positive relationship between factor V Leiden mutation and fetal loss (odds ratio: 10.8). FVL mutation is a common genetic defect and its prevalence was reported as four percent in Caucasians and 4.3% in the Greek population [14, 15].

A meta-analysis reported an odds ratio of 2.0 in terms of association between factor V Leiden and factor II mutations and RPL [16]. In their study on Jewish women, Brenner *et al.* reported that frequency of FVL and factor II mutations were significantly higher in their study group compared to controls (32% - 10% and 8% - 4%, respectively) [17]. In a study performed in Greek population, Foka *et al.* reported significantly higher frequencies of FVL and FII mutations in their study group compared to controls (19% - 4%, $p = 0.003$, OR = 5.5 vs 9%- 2%, $p = 0.038$, OR = 4.6) [18]. In terms of association between FVL and FII mutations and RPL, a study performed by Settin *et al.* revealed odds ratios of 21.38 vs 36.7, respectively [19]. In the present study, the authors found an odds ratio of 10.8 in terms of association between FVL and RPL (21.7%-2.5%, $p = 0.007$, odds ratio = 10.8). Although FII gene mutation prevalence was high in the study group, since there was no case with FII gene mutation in the study group, odds ratio calculation was unavailable (10% - 0%, $p = 0.039$).

Zammiti *et al.* and Mtiraoi *et al.* reported relatively higher FVL mutation rates in their study groups compared to controls, however FII mutation was not significantly higher in Tunisian patients [20, 21]. Also, Grandone *et al.* reported higher FVL mutation rates in affected Italian women (16.28% - 4.24%, $p = 0.011$) [22]. On the contrary to the above studies, in their study on Turkish women with RPL, Sehirali *et al.* observed more significantly higher frequencies of FII mutations than FVL mutations in their study group compared to controls [23]. Meanwhile, some studies found no association between RPL and FVL and FII mutations [12, 24-29].

In a meta-analysis, Rey *et al.* reported a positive relationship between FVL mutation and both early and late pregnancy losses, while they found FII mutation was relatively more associated with early pregnancy losses [30]. Reznikoff-Etiévant *et al.*, in their study which included 260 Caucasian women with two or more concomitant pregnancy losses before ten weeks gestation, reported that FVL mutation was significantly associated with RPL before ten weeks gestation [31]. Krause *et al.* also reported significantly high frequencies of FVL mutations in German women with early pregnancy losses [32]. Meanwhile, some studies reported that FVL mutation was found in higher frequencies among cases with late pregnancy losses [22, 33, 34]. There are many sudies which present evidence of a strong association between FII gene mutation and late pregnancy losses [17, 32-35]. In the present study, the authors found that FVL mutation was more frequent in cases with early pregnancy loss (23.9%-2.5%, $p = 0.004$, odds ratio: 12.26). Although prevalance of FVL mutation was higher in cases with late pregnancy loss, it did not reach statistical significance (14.3%-2.5%, $p = 0.09$, odds ratio: 6.5). FII mutation was also found as more frequent in cases with early RPL, however odds ratio could not be calculated since there were no cases with FII gene mutation in women in the control group (10.87%-0%, $p = 0.032$). FII gene mutation prevalance was also higher in cases with late RPL, however it was statistically insignificant (7.14% - 0%, $p > 0.05$).

Kutteh *et al.* found no statistical difference between groups with primary and secondary RPL in terms of FVL mutation prevalence [36]. In the present study, while FVL mutation prevalence was significantly higher in the group with primary RPL (29.3% - 2.5%, $p = 0.001$, odds ratio = 16.14), it was higher but statistically insignificant in the group with secondary RPL compared to the control group (5.3%-2.5%, $p = 0.3$).

MTHFR deficiency is a metabolic disease, which is thought to cause placental infarcts associated with arterial and venous thromboemboli, has been studied in women with RPL. Brenner *et al.* and Kutteh *et al.* reported no association between MTHFR C677T gene mutation homozygosity and RPL [17, 36]. In their meta-analysis, Rey *et al.* found no significant association between MTHFR mutation homozygosity and RPL [30]. Habibovic *et al.* reported no significant association between MTHFR C677T gene mutation and RPL, as a result of their study on Turkish population [37]. The present authors also did not find any significant association between MTHFR gene mutation and RPL (48.3%-42.5%, $p = 0.566$, odds ratio = 1.27).

Conclusion

Results of the present study showed that RPL was associated with FVL and FII gene mutations but not with MTHFR mutation. The results also propose that FVL and FII gene mutations may be predisposing factors for RPL, especially for early and primary RPL.

In addition, the present study revealed no significant difference between cases with three or more RPL and cases with only two concomitant pregnancy losses, in terms of FVL and FII gene mutation prevalence.

Paucity of cases included to the control group was a limitation of this study. In the literature, there is no consensus on the association between RPL and thrombophilic factors. Thus, properly-designed further studies which include larger numbers of women are needed to illuminate this subject.

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Address reprint requests to:
U. ISAOGLU, M.D.
Nenehatun Obstetrics and Gynecology Hospital
Erzurum (Turkey)
e-mail: u.isaoglu@gmail.com