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Prevalence of thromogenic gene mutations in women with recurrent miscarriage: A retrospective study of 1,507 patients

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Objective
Thromogenic gene mutations has been thought to be associated with recurrent pregnancy loss in women in Turkey. The aim of this study was to investigate the prevalence of thromogenic gene mutations such as factor V Leiden (FVL, G1691T), prothrombin (G20210A), and the methylene tetrahydrofolate reductase (MTHFR, C677T) mutation in women with recurrent pregnancy loss.

Methods
This descriptive study was carried out in the Department of Obstetrics and Gynaecology, Harran University School of Medicine, and included a total of 1,507 women with histories of recurrent pregnancy loss between January 2010 and June 2013. The mutations were assessed by using the polymerase chain reaction.

Results
The homozygous mutation frequencies of FVL, prothrombin, and MTHFR were found to be 3 (0.20%), 0 and 125 (8.29%), and the heterozygous mutation frequencies were 83 (5.51%), 61 (4.05%), and 612 (40.61%), respectively. Among the 86 FVL mutation patients, 38 also had accompanying prothrombin and MTHFR mutations.

Conclusion
Since the homozygous forms of the FVL—prothrombin gene mutations have low incidences and MTHFR mutation is similar to a healthy population, preconceptional thromogenic gene mutations screening seems to be controversial.

Keywords: Factor V Leiden; Methylenetetrahydrofolate reductas; Prevalence; Prothrombin; Recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL) is described as three or more consecutive spontaneous early (<12 weeks gestation) miscarriages [1]. It is a common obstetric disease which is challenging for both the patients and obstetricians, and approximately 2% of reproductive-aged women suffer from this disease worldwide [2]. It has been proposed that a large number of etiological factors, such as uterine abnormalities, endocrine problems, immunological abnormalities, chromosomal abnormalities and infectious diseases have been associated with recurrent pregnancy losses [3,4]. In women with RPL who do not have the above-mentioned factors, it has been suggested that thrombophilia may be the underlying pathology [4].

Thrombophilia is a condition predisposing one to hypercoagulability, which may be acquired or inherited. The most common causes of inherited thrombophilia are thromogenic gene mutations such as factor V Leiden (FVL) mutation, prothrombin gene

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mutation and methylene tetrahydrofolate reductase (MTHFR) mutation [1,2]. Although there are many studies showing the relationships between thrombophilia and RPL [5-7], there is still debate as to whether thrombophilia is the cause of, or merely associated with, recurrent pregnancy loss [1,8].

In this study, the frequencies of these three mutations in patients who were admitted to the Obstetrics and Gynaecology Clinic of Harran University School of Medicine, with complaints of RPL, were collected retrospectively.

Materials and methods

This retrospective study was carried out in the Department of Obstetrics and Gynaecology at the Harran University School of Medicine. This study design was in accordance with the guidelines of the Declaration of Helsinki (second revision, 2008), and was approved by the local ethics committee.

The records of 1,566 patients were achieved for two and a half years period. The patients who have uterine anomalies (7 patients) and endocrine dysfunctions (52 patients) were not included in the study. This study included a total of 1,507 women who were admitted to the infertility clinic with desires to increase fertility, and with histories of RPL, between January 2010 and June 2013.

RPL is described as three or more early (<12 weeks gestation) consecutive miscarriages. The gestational age for miscarriages was calculated according to the last menstrual date, and the first available ultrasound.

1. Blood collection

All blood samples were obtained in the morning, when the patients were fasting, from the antecubital vein without inhibiting the venous blood flow, using plastic syringes. The total sample volume was 10 mL. Five millilitres of the samples were aliquoted for laboratory tests, and the remaining 5 mL was mixed with 25 cc of red blood cell lysing solution (155 mM ammonium chloride [AppliChem, Darmstadt, Germany], 10 mM sodium bicarbonate [Merck, Darmstadt, Germany], 0.5 mm EDTA [AppliChem]). This mixture was allowed to stand on ice for 20 minutes and then centrifuged at 4,000 rpm at 4°C for 20 minutes. After this, the supernatant was poured off and 25 cc of the red blood cell lysing solution were added to the pellet again. This process was repeated until all red blood cells were removed. The isolated DNA was stored at -70°C until further analysis.

2. Detection of factor V Leiden, prothrombin gene and methylene tetrahydrofolate reductase mutation

The FVL, prothrombin, and MTHFR mutations were determined using the FV-PTH-MTHFR Strip A kit (Vienna Lab, Vienna, Austria), which can detect FVL, prothrombin, and MTHFR gene mutations at the same time. This molecular analysis was based on the hybridization of polymerase chain reaction-amplified DNA products, with mutation-specific oligonucleotide probes (reverse dot blot) [9].

Results

The mean age of the patients included in this study was 24.69±2.48 years (range, 19–34 years). The mean number of pregnancy losses was determined to be 3.16±0.41 (range, 3–7). For all patients, the FVL, prothrombin gene and MTHFR mutations were analysed as a screening test for thrombophilia. The results of the screening were summarized in Table 1. When looking for FVL (G1691T) mutation, eighty-three patients (5.51%) were heterozygous, and 3 patients (0.20%) were homozygous. The frequency of heterozygous prothrombin gene mutation (G20210A) was determined to be 4.05% (61/1,507). We did not encounter a homozygous mutation of this gene. The frequency of heterozygous MTHFR (C677T) was identified as 40.61% (612 of 1507 patients) and homozygous mutation were 8.29% (125 of 1,507 patients). In conjunction

Table 1. The frequency of thrombophilia in women with recurrent pregnancy loss

	Heterozygous	Homozygous	Normal
FVL analysis	83 (5.51)	3 (0.20)	1,421 (94.29)
Prothrombine analysis	61 (4.05)	None	1,446 (95.95)
MTHFR analysis	612 (40.61)	125 (8.29)	737 (48.90)

Values are presented as number (%).
FVL, factor V Leiden; MTHFR, methylene tetrahydrofolate reductase.

with FVL, other thromogenic gene mutations were found in 38 out of 86 patients: 33 patients had MTHFR and heterozygous FVL (2.19%) mutations and 5 patients had prothrombin G20210A and heterozygous FVL (0.33%) mutations.

Discussion

Thrombophilias are a group of various coagulation disorders related to a predisposition for thrombotic events (e.g., deep vein thrombosis and pulmonary embolism). It is proposed that pregnancy loss is associated with inherited thrombophilia. Although the pathophysiology is complex, interrelated thrombosis leading to placental insufficiency, and the inhibition of trophoblast differentiation, is thought to be responsible. The most common causes of inherited thrombophilia include FVL, prothrombin and MTHFR mutations [1,3,8,10].

FVL mutations occur with the replacement of adenine by guanine at the 1691 gene position, with factor V becoming resistant to the action of activated protein C [10]. As a consequence, an increase in the hypercoagulable state during pregnancy can lead to complications such as pregnancy loss and RPL [1,3]. Wide variations in the heterozygous frequencies of FVL have been reported in different surveys carried out in many countries, and the highest prevalency rates were determined to be in the Mediterranean countries, such as Lebanon (14.4%), Cyprus (12.1%), and Jordan (12.3%). There were no detected mutations in certain populations, such as Japanese, Chinese, African, and Native Americans [9]. The broad variations in the prevalence of FVL have been found in several research studies performed in different regions of Turkey, which is also a Mediterranean country. The heterozygous frequency of the FVL mutation in Turkey was found to be 7.9%. While the highest prevalence rates in Turkey were in the cities of Ankara (9.8%) and Istanbul (10.3%), the lowest frequency was observed in the Thrace region (4.28%) [9,11,12]. The prevalence of the FV Leiden mutation in Diyarbakir, which neighbours Sanliurfa, has been found to be 4.6% [13]. In our study, the frequency of FVL carriers was 5.51%. This rate was lower than the national prevalence, but was higher in Diyarbakir. We speculate that this result is due to the geographical localization of Diyarbakir, in which the population has many immigrants from southeastern Anatolia.

The prothrombin gene mutation is the result of a G-to-A substitution at position 20210 in the 3′ untranslated region, and it causes an increase in the level of serum prothrombin. This elevation can cause an increased risk for venous thrombosis, arterial disease and RPL [14]. The heterozygous prothrombin G20210A mutation was identified in between 1.2% to 2.7% of individuals in Turkey [9,15]. The prevalence of the heterozygous prothrombin gene mutation in Sanliurfa, which is in the southeastern part of Anatolia, has been found to be 1.7% (two out of 114) [16]. In our study, the frequency of the heterozygous prothrombin gene mutation was 4.05%. This rate was higher than the national prevalence and that of southeastern Anatolia. We believe that this difference may be related to the study population size difference.

The MTHFR mutation is the change of C to T at position 677. As a result of this mutation, enzyme activity is reduced and subsequently results in a homocysteine accumulation in the blood [2,14,17]. It has been suggested that high levels of homocysteine could be one possible reason for RPL [2]. The frequencies of heterozygosity and homozygosity for MTHFR (C677T) were determined to be 47.4% and 9.6%, respectively, in a healthy population from Turkey [18]. Yildiz et al. [19] studied the prevalence of thromogenic gene mutations in RPL patients in Turkey and found the MTHFR C677T gene mutation to be lower than in the control group (36.9% heterozygous, 3.5% homozygous in PRL patients, in the control group 42.6% heterozygous and 2.1% homozygous). In our study, which included 1,507 RPL patients, the frequency of heterozygous and homozygous MTHFR mutations was found to be 40.61% and 8.29%, respectively. These ratios were lower than the national prevalence, but greater than the ones in Yildiz's study. This difference may be related to the study group size.

There is still debate about whether thromogenic gene mutations is the reason for, or only associated with, recurrent pregnancy loss. While several authors have considered the relationship between thrombophilia and recurrent pregnancy loss [2,5,7], some authors did not find such a relationship [1,20,21]. Moreover, it is suggested that inherent thrombophilias are not one of the reasons for RPL and cannot affect the live birth rate in women with RPL [22,23].

Screening tests for thromogenic gene mutations prior to pregnancy are controversial. The FVL and prothrombin gene mutations have a low frequency in the population, and women with these mutations can have normal pregnancies, therefore, it is suggested that the effect of these mutations on pregnancy loss is minimal [24]. The ACOG Practice Bulletin in "Inherited thrombophilias in pregnancy" did not propose screening tests in women with RPL, due to the fact that it is

not definite that anticoagulation decreases future miscarriages [25]. The screening tests for thrombophilias are very expensive, therefore, these tests are only recommended for patients who have deep vein thromboses and pregnancy complications [26]. In our opinion, since the homozygous forms of the FVL—prothrombin gene mutations have low incidences and MTHFR mutation is similar to a healthy population, preconceptional thrombophilia screening seems to be controversial.

The present study has some limitations, summarized as follows: first, it is designed as a retrospective study. A second limitation, the causes of RPL such as parental chromosome abnormality, autoimmune causes (antiphospholipid antibody syndrome, systemic lupus erythematosus) and alloimmune cause (high natural killer cell) did not be evaluated in this study.

RPL is a challenge for obstetricians, and so far no definitive cure has been described for this problem. During the last decade, thromogenic gene mutation screening was presented as a promising method; however, our results have demonstrated that the incidence of thromogenic gene mutation is not as high as predicted. Until more effective tests can been found, thromogenic gene mutation screening seems to maintain its popularity.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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