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INCIDENCE AND SPECTRUM OF THROMBOPHILIA IN WOMEN WITH RECURRENT PREGNANCY LOSS: A RETROSPECTIVE STUDY

TEKRARLAYAN GEBELİK KAYBI OLAN KADINLARDA TROMBOFİLİ İNSİDANSI VE SPEKTRUMU: RETROSPEKTİF BİR ÇALIŞMA

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ABSTRACT

Objective: At least 2 pregnancy losses before the 20th week of pregnancy Recurrent pregnancy loss (RPL), known as recurrent pregnancy loss (RPL), occurs in 3 to 5% of women. The aim is to evaluate the frequency of common variants with hereditary thrombophilia; (Methylenetetrahydrofolate Reductase; MTHFR C677T and MTHFR A1298C polymorphisms; FV Leiden G1691A and Prothrombin G20210A mutation) in female patients with RPL.

Materials and Methods: This retrospective cohort study is between the 2020 and 2022 years. We investigated the frequency of the MTHFR C677T and MTHFR A1298C polymorphisms, FV Leiden G1691A and Prothrombin G20210A mutations in 380 females suffering from two or more pregnancy losses.

Results: The mean age of the patients was 32.6 years (range: 18-46). The most frequently observed variant in this group was a heterozygote mutation for the MTHFR677 polymorphism (n=163, 42.8%). The second most common variant was MTHFR A1298C heterozygosity with a frequency of 41.1% (n=156). The least common variant is FII homozygosity (0.3%). Heterozygous of FII and FV Leiden (G1691A) mutations were found in 1.8% (1/380) and 8.4% (7/380) of the patients, respectively.

Conclusion: The thrombophilic variants (FV Leiden G1691A and Prothrombin G20210A) seem to have an important role in RPL. Detection of thrombophilia in couples suffering from RPL can be important for initiating early and appropriate treatment.

Keywords: Factor V Leiden, hereditary thrombophilia, Prothrombin, retrospective study, recurrent pregnancy loss

ÖZET

Amaç: Gebeliğin 20. haftasından önce en az 2 gebelik kaybı yaşanması durumu olarak bilinen tekrarlayan gebelik kayıpları (TGK) kadınların %3 ila 5'inde görülmektedir. Amaç, RPL'li kadın hastalarda kalıtsal trombofili ile sık görülen varyantların (Metilentetrahidrofolat Redüktaz; MTHFR C677T ve MTHFR A1298C polimorfizmleri; FV Leiden G1691A ve Protrombin G20210A mutasyonu) frekansını değerlendirmektir.

Gereç ve yöntem: Bu retrospektif kohort çalışması 2020 ile 2022 yılları arasındadır. İki veya daha fazla gebelik kaybı olan 380 kadında MTHFR C677T ve MTHFR A1298C polimorfizmleri, FV Leiden G1691A ve Protrombin G20210A mutasyonlarının sıklığını araştırdık.

Bulgular: Hastaların ortalama yaşı 32.6 yıl (dağılım: 18-46) idi. Bu grupta en sık gözlenen varyant, MTHFR677 polimorfizmi için bir heterozigot mutasyonuydu (n=163, %42.8). İkinci en yaygın varyant, %41.1 (n=156) sıklık ile MTHFR A1298C heterozigotluğuydu. En az yaygın varyant ise FII homozigotluğuydu (%0.3). Heterozigot FII ve FV Leiden (G1691A) mutasyonları sırasıyla hastaların %1.8'inde (1/380) ve %8.4'ünde (7/380) bulundu.

Sonuç: Trombofilik varyantların (FV Leiden G1691A ve Prothrombin G20210A) RPL'de önemli bir rolü olduğu görülmektedir. TGK'dan muzdarip çiftlerde trombofilinin tespiti erken ve uygun tedaviye başlamak için önemli olabilir.

Anahtar Kelimeler: Faktör V Leiden, kalıtsal trombofili, Protrombin, retrospektif çalışma, tekrarlayan gebelik kayıpları

INTRODUCTION

The spontaneous abortion of two or more consecutive pregnancies in the trimester of 1st or 2nd is defined as recurrent pregnancy loss (RPL). About 3-5% of all pregnancies result in RPL (1, 2). Chromosomal abnormalities, anatomical changes, infections and endocrinological dysfunctions must be excluded in women with RPL. However, the pathogenesis of RPL remains unknown in many cases. Previous studies showed an association between congenital or acquired thrombophilia and RPL (3). RPL is a predictable complication in a hypercoagulable state. Prothrombin G20210A and Factor V Leiden mutations and immunologic disorders such as antithrombin III, Protein S and Protein C deficiency are common in women with RPL. The role of MTHFR polymorphisms in RPL is not fully elucidated, and the results of some of these reports suggest a possible association between RPL (3-5). In a study conducted in Turkey, MTHFR polymorisms were found to be more common in women who had 3 or more miscarriages compared to healthy controls (5). According to the literature, there are many studies that have determined that there is no relationship between MTHFR gene polymorphisms and RPL (2-4). In this study, we evaluated the frequency of common thrombophilic

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variants (MTHFR gene polymorphisms; FV Leiden, and FII gene mutations) in women with RPL.

MATERIAL AND METHODS

The retrospective study was performed between October 2020 and January 2022. The 380 women (18-46 years old) who applied to Eskişehir City Hospital Obstetrics and Gynecology outpatient clinic due to RPL (more than two abortions before 20th gestational week) were included. The study was approved by the Ethics Committee of Eskisehir Osmangazi University (2022-110/41). Each participant filled an informed consent form. DNA isolation was performed from blood samples taken into tubes containing ethylene diamine tetra acetic acid (EDTA). Genomic DNA was isolated from individuals by using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Germany). To determine the prevalence of the mutations in THE CVD panel, the subjects were genotyped for Factor V Lieden, Factor II (Prothrombin), MTHFRA1298C and C677T mutations by using Real-time PCR with Cobas Z 480 LightCycler (Roche Molecular Diagnostics). Factor V Leiden Kit (RocheMolecular Systems) and Factor II G20210A Kit (Roche MolecularSystems, Branchburg, New Jersey, USA) were used for Factor V and FII mutations, respectively. For MTHFRA1298C and MTHFR C677T, LightCycler FastStart DNA Master mix was used containing specific primers and probes for each of them, respectively. We investigated the frequency of common thrombophilic variants (MTHFR C677T and MTHFR A1298C polymorphisms; FV Leiden G1691A and Prothrombin G20210A mutation) calculated for this retrospective study. Statistical analyzes were performed using SPSS 10.0 software. All clinical data obtained in this analysis were expressed as a percentage.

RESULTS

A total of 380 females were included in the study. The mean age of the paticipatiants was 32.6 years (range: 18-46). The mean numbers of gravida, parity and abortion were found as 3.64±1.24, 0.96±1.56, and 3.23±1.08, respectively. Demographic and clinical characteristics of the sample were listed in Table 1. The study examined four common thrombophilia factors in the patient group; MTHFR C677T and A1298C polymorphisms, FV Leiden G1691A and Prothrombin G20210A (Factor II) mutations. There was no variant in 70 (18.4%) of the patients. The most common variant in this group was MTHFR 677 heterozygosity (n=163, Table 2). The second common variant was the MTHFR A1298C heterozygosity with a 41.1% frequency (n=156). The frequency of FV Leiden heterozygosity was 8.4%. In 4 of 380 patients (1.1%), the Factor V Leiden mutation was found to be homozygous. The frequency of heterozygous Factor II mutation was 1.8%. Factor II homozygosity was detected in only one patient (0.3%, Table 2). Allele frequencies of FV Leiden mutation were determined to be 94.8% for the G allele and 5.2% for the A allele. The allele frequencies of FII were 98.8% for the G allele and 1.2% for the A allele, respectively. Allele frequencies of MTHFR C677T polymorphism were detected at 70.6% for the C allele and 29.4% for the T allele. In addition, the allele frequencies of MTHFRA1298C polymorphism were found to be 68.4% and 31.6% for A and C alleles, respectively (Table 3). When the association of two genetic changes was examined, MTHFR C677T and MTHFR A1298C coincidences were observed most third frequently with 32.6% frequency (n=124, Table 4). The next most common two mutations togetherness were FV Leiden-MTHFR A1298C and FV Leiden-MTHFR C677T with 3.2% (n=12) and 4.8% (n=18) frequency (Table 4). Only five patients were found to carry the FV Leiden, MTHFR C677T and MTHFR A1298C variants (1.3%). Then; MTHFR 677, MTHFR 1298 and FII coincidence were detected in 2 patients with a 0.5% frequency (Table 4).

Table 1. Demographic characteristics of the patient group.

Demographic characteristics	Mean±SD	Min-Max
Age	32.6 ±7.8	18-46
Gravida (n)	3.64±1.24	2-9
Parity (n)	0.96±1.56	0-4
Abortion (n)	3.23±1.08	2-7

Note: This table displays descriptive statistics for each data. The statistics estimated are mean, minimum, maximum and standard deviation (SD).

Table 2. The genotype frequencies of FV Leiden G1691A, Prothrombin G20210A, MTHFR C677T and MTHFR A1298C.

Genotypes frequencies	**Case (n=380) (%)**
FVL G1691A	**N (%)**
GG	344 (90.5)
GA	32 (8.4)
AA	4 (1.1)
Prothrombin G20210A	**Case (n=380) (%)**
GG	372 (97.9)
GA	7 (1.8)
AA	1 (0.3)
MTHFR C677T	**Case (n=380)(%)**
CC	187 (49.2)
CT	163 (42.9)
TT	30 (7.9)
MTHFR A1298C	**Case (n=380)(%)**
AA	182 (47.8)
AC	156 (41.1)
CC	42 (11.1)

Table 3. The allele frequencies of FV Leiden G1691A, Prothrombin G20210A, MTHFR C677T and MTHFR A1298C.

Allele frequencies	**Case (n=380) (%)**
FVL G1691A	**N (%)**
G	720 (94.8)
A	40 (5.2)
Prothrombin G20210A	**Case (n=380)**
G	751 (98.8)
A	9 (1.2)
MTHFR C677T	**Case (n=380)**
C	537 (70.6)
T	223 (29.4)
MTHFR A1298C	**Case (n=380)**
A	520 (68.4)
C	240 (31.6)

Table 4. Distribution of genotype frequencies of patients with complex variants.

Genotype frequencies	**Case (n=380) N (%)**
MTHFR C677T + MTHFR A1298C (CT+AC)	124 (32.6)
FVL G1691A + MTHFR A1298C (GA+AC)	18 (4.8)
FVL G1691A + MTHFR C677T (GA+CT)	12 (3.2)
FVL G1691A + MTHFR C677T + MTHFR A1298C (GA+CT+AC)	5 (1.3)
Prothrombin G20210A + MTHFR C677T + MTHFR A1298C (GA+CT+AC)	2 (0.5)

DISCUSSION

The causes of RPL include many factors, including anatomical, endocrine disorders, antiphospholipid syndrome, and chromosomal abnormalities. The causes of 30-50% of RPL cases cannot be clarified. Recently, thrombophilia factors have been shown to be among the possible causes of RPL by causing thrombosis. Hereditary thrombophilia can be linked for many complications of obstretry such as recurrent pregnancy loss, infertility, preeclampsia, fetal growth retardation and stillbirth (6-8). This study was designed to show the frequencies of MTHFR (C677T and A1298C) polymorphisms, and FV Leiden and Prothrombin mutations among the 380 females with RPL. FV and FII mutations are the two most common inherited thrombophilia factors (7). The Factor V (G1691A) mutation, which causes a structural change in the Factor V molecule, makes it less susceptible to inactivation by active protein C (APC). Factor V mutation has been shown as the most common inherited thrombotic risk factor associated with RPL. Factor V (G1691A) mutation might be a significant risk factor, with a reported incidence of 0.5–18% in patients and 4–10% in control groups (9-12). In our study, we detected AA homozygosity in 4 of 380 (1.1%) patients, and we found the A allele frequency at 5.2%. The G to A change in the 3'-untranslated region of the prothrombin gene in 20210 upsets the balance of hemostasis in favor of coagulation susceptibility. There are several studies showing that the prothrombin A20210G mutation poses a risk for early RPL (13, 14). We revealed that we found wild GG genotype with a frequency of 97.9%, heterozygous GA genotype with a frequency of 1.8% and homozygous (mutant) AA genotype with a frequency of 0.3% of the prothrombin G20210A mutation. The prevalence of factor II heterozygous in the Turkish population varies between 1.37 and 4%, with an overall prevalence of about 2% (15). Similarly, in our study, we detected the Factor II heterozygosity as 1.8%. MTHFR is an important enzyme which is involved in the DNA synthesis and homocysteine metabolism. The MTHFR 677C>T polymorphism causes a defective methylene tetrahydrofolate reductase enzyme, resulting in high homocysteine levels (7, 13, 16). Plasma homocysteine levels are found to be slightly higher in homozygous MTHFR C677T gene mutations compared to the general population, and this may cause a 3-fold increase in the risk of VTE (17). The C677T polymorphism has been linked with an increased 3-fold risk of RPL (18). There are still conflicting results regarding the relationship between MTHFR polymorphisms and RPL (19). In this study, the 677 CC, 677 CT and 677 TT genotypes were found at frequencies of 49.2%, 42.9% and 7.9% in females with RPL. The TT genotype of MTHFR677 in healthy individuals varies between populations. The frequency of the TT genotype was 1.9% in North Americans, 11.6% in Europeans, 5.5% in Asians, 2.4% in Africans and 5.9% in Australians (20). The MTHFR 1298A > C polymorphism in exon 7 is a highly prevalent polymorphism in various populations (1-12%) (21, 22). In this study, the frequency of homozygotes for 1298 AA was 47.8% and for 1298 CC was 11.1% while the frequency of heterozygotes for 1298 AC was 41.1%. The prevalence of the alleles A and C were 68.4% and 31.6%, respectively. In our study, the most common genotypic variation was in the MTHFR region in 42% of the cases. The two most common polymorphisms in the MTHFR gene are C677T and A1298C, and they are associated with low MTHFR activity, and high homocysteine levels (23). The mutant alleles of MTHFR C677T and A1298C were demonstrated to be higher in a Turkish study with RPL individuals (24). At least one abnormality in terms of these gene polymorphisms was detected in 310 (81.6%) of the 380 women included in this study, and the most

common of these was MTHFR677 heterozygosity (42.9%). The least common variant is FII homozygosity (0.3%). The limitation of this study is the absence of a control group. Therefore, we compared our results to statistics found in other studies.

CONCLUSION

Although the role of hereditary thrombophilia in the etiology of recurrent pregnancy loss has been accepted, it is not clear to what extent hereditary thrombophilia is involved. The most common studies on the prevalence of hereditary thrombophilias concern the FV Leiden, Prothrombin and MTHFR gene variants. Considering the results of this and other studies, it was thought that these three gene variants were insufficient to explain the cause of RPL and may be due to the combined coexistence of mutations of thrombophilia-related genes rather than a specific mutation. Therefore, future studies are needed to investigate the prevalence of genes that cause hereditary thrombophilia in women with RPL.

Ethics Committee Approval: The study protocol was approved by the ethical committee of Eskisehir Osmangazi Medical Faculty (Protocol Number: 2022-110).

Informed Consent: This study was done retrospectively.

Authorship Contributions: Idea/Concept: AK, Design: AK, Supervision: AK, HK, SG, Data Collection or Processing: AK, HK, SG, Analysis or Interpretation: AK, Literature Search: AK, Writing: AK, Critical Review: AK, HK, SG, References And Fundings: -, Materials: AK.

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