

The association of factor V G1961A (factor V Leiden), prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G polymorphisms with recurrent pregnancy loss in Bosnian women

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ABSTRACT

Aim To investigate association of factor V Leiden, prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G-polymorphisms with recurrent pregnancy loss in Bosnian women.

Methods A total of 60 women with two or more consecutive miscarriages before 20 weeks of gestation with the same partners and without history of known causes or recurrent pregnancy loss were included. A control group included 80 healthy women who had one or more successful pregnancies without history of any complication which could be associated with miscarriages. Genotyping of factor V Leiden, prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G polymorphisms were performed by polymerase chain reaction/restriction fragments length polymorphism method (PCR/RFLP).

Results Both factor V Leiden and MTHFR C677T polymorphisms were significantly associated with recurrent pregnancy loss (RPL) in Bosnian women while prothrombin G20210A and PAI-1 4G/5G polymorphisms did not show strongly significant association.

Conclusion The presence of thrombophilic polymorphisms may predispose women to recurrent pregnancy loss. Future investigation should be addressed in order to find when carriers of those mutations, polymorphisms should be treated with anticoagulant therapy.

Key words: thrombophilia, habitual abortions, gene polymorphisms

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INTRODUCTION

Thrombophilia represents acquired and/or genetic condition that predisposes individuals to venous and arterial thrombosis (1). Recently, hereditary thrombophilia has gained a lot of attention as a risk factor for pregnancy complications (2). Pregnancy is a physiological hypercoagulable state and it is known that disturbance in coagulation cascade may contribute to serious pregnancy complications such as preeclampsia, foetal growth restriction, placental abruption and pregnancy loss (2). Recurrent miscarriage is a common health problem and is defined as two or more pregnancy losses before 20 weeks of gestation (3,4).

The most extensively studied inherited thrombophilic factors that could be involved in recurrent miscarriage are the polymorphisms of G20210A prothrombin gene, G1691A of factor V gene (Factor V Leiden) and 4G/5G polymorphism of PAI-1 gene (5).

Factor V Leiden is common mutation G→A in the nucleotide position 1691 in the factor V gene and causes activated protein C (APC) resistance, which accounts for most cases of venous thrombosis and recurrent miscarriage (6,7).

A central factor of coagulation cascade is coagulation factor II or thrombin coded by prothrombin gene. Its precursor is prothrombin which is cleaved and thus activated through the action of coagulation factor X (8). The clinical expression of prothrombin-related thrombophilia is variable (9). It may increase the risk for pregnancy loss but many individuals heterozygous or homozygous for the 20210G>A polymorphism never develop thrombosis, while most heterozygotes who develop thrombotic complications remain asymptomatic until adulthood (9). There is a number of studies showing an increased risk of recurrent miscarriage in women with inherited factor V Leiden and prothrombin G20210A polymorphisms (10-12).

The MTHFR C677T polymorphism is commonly associated with hyperhomocysteinemia and it results in an alanine to valine substitution in the predicted catalytic domain of the enzyme (13). Through its effect on total homocysteine levels, MTHFR C677T polymorphism has been implicated as a risk factor in the pathogenesis of recurrent miscarriages (14). This was highlighted by findings that the prevalence of homozygous variants of MTHFR C677T polymorphism was

higher among women with more than three idiopathic recurrent miscarriages (15).

Type I plasminogen activator inhibitor (PAI-1) is the predominant inhibitor tissue and urinary type activator. The commonly studied functional variant in the PAI-1 gene is 1-bp guanine insertion/deletion polymorphism at position -675 nucleotides relative to the transcription start site (16). According to role of PAI -1 gene in fibrinolysis it was suggested that it is involved in etiology of intrauterine foetal death, intrauterine growth restriction, preterm placental abruption, recurrent miscarriage and preeclampsia (17).

The aim of this study was to investigate association of factor V Leiden G1961A, prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G polymorphisms with recurrent miscarriage in a group of women of the Bosnian population. This research was motivated by lack of information on this topic in Bosnia and Herzegovina.

PATIENTS AND METHODS

Patients and study design

This prospective study included 60 women attended to the IVF Centre Dr Balic Tuzla, the Gynaecology Polyclinic 'Korak do života' Tuzla, and Gynaecology and Obstetrics Department of the University Clinical Centre Tuzla during the period between September 2014 and February 2017.

Inclusion criteria were two or more consecutive miscarriages before 20 weeks of gestation with the same partners and without history of known causes or recurrent pregnancy loss (RPL) (chromosomal defect, chronic disease, infection, hormonal impairment, thromboembolic disease and other anatomical anomalies and complications of genitourinary tract which could cause miscarriage).

Four of 60 women (median age 33.05 years, range 17-45 years) had two or three pregnancy losses after ten weeks of gestation, 56 had pregnancy loss before ten weeks.

The control group consisted of 80 healthy women attended to the same institutions for regular follow up (median age 34.08 years, range 19-55 years) who had one or more successful pregnancies without history of any complications which could be associated with miscarriages.

All cases and controls were fully informed about the study protocol by the main investigator and

have agreed to participate in the study by signing the written consent. The study was approved by the Ethics Committee of the University Clinical Centre Tuzla.

Methods

Genomic DNA was extracted from blood using the commercial FlexiGene DNA Isolation Kit (250) (Qiagen GmbH, Hilden, Germany) in the Laboratory of Genetic, Biology Department of the Faculty of Natural Sciences and Mathematics, University of Tuzla. Genotyping of factor V Leiden, prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G polymorphisms was performed by polymerase chain reaction/restriction fragments length polymorphism method (PCR/RFLP) according to previously described protocols (18-21). The genotypes were determined by electrophoresis in 4.0% agarose gel (Sigma Aldrich Chemie GmbH, Munich, Germany) stained with Ethidium bromide (Sigma Aldrich Chemie GmbH, Munich, Germany).

Statistical analysis

Frequencies of allele and genotypes were compared by χ^2 test. For all mutations, the odds ratio (OR) and their 95% confidence intervals (CI) were calculated according to McHugh (22) to estimate the risk for recurrent miscarriage for carriers of investigated polymorphisms. Statistical significance was set as $p < 0.05$.

RESULTS

The allele frequencies of factor V Leiden were significantly higher in RPL patients than in controls, 7.5% and 3.75%, respectively ($p=0.021$). The mutant homozygous carriers of FVL were not identified. The presence of A allele of FVL was associated with four times higher risk of recurrent miscarriage in RPL patients [OR (95% CI) =4.243 (1.123 – 16.029)] ($p=0.033$).

Distribution of allele A of prothrombin G2021A was not significantly different in RPL patients, 2.5%, than in controls, 0.63% ($p=0.190$). The risk of RPL was four times higher in carriers of A allele of prothrombin G2021A polymorphism [OR (95 CI) =4.076 (0.418 – 39.690)] ($p=0.226$).

Higher frequencies of T allele of MTHFR C677T polymorphism in RPL patients than in controls, 39.1% and 25%, respectively, ($p=0.016$) were found, and its association with recurrent miscarriage is nearly two times higher than C allele [OR (95 CI) =1.772 (1.074 – 2.925)] ($p=0.025$).

In this study we did not find an association between 4G/5G and 4G/4G genotypes of PAI-I 4G/5G polymorphism with recurrent pregnancy loss in Bosnian population [OR (95 CI) =1.559 (1.790 – 3.075)] ($p=0.199$). The frequencies of 4G allele and 5G allele were not significantly different between RPL patients and controls ($p=0.073$) (Table 1).

Table 1. Allele and genotype frequencies of factor V Leiden (FVL), prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G-polymorphism

| Gene polymorphism | Genotypes and Alleles | RPL n (%) | Controls n (%) | OR* (95% CI) | p |
|---------------------|-----------------------|-------------|-----------------|------------------|-------|
| FVL (G1961A) | Wild type G/G | 51 (85%) | 77 (96.25%) | 4.529 | 0.028 |
| | Heterozygous G/A | 9 (15%) | 3 (3.75%) | (1.169 – 17.537) | |
| | Homozygous A/A | - | - | - | |
| Prothrombin G20210A | Allele G | 111 (92.5%) | 157 (98.12%) | 4.243 | 0.033 |
| | Allele A | 9 (7.5%) | 3 (1.88%) | (1.123 – 16.029) | |
| | Wild type G/G | 57 (95%) | 79 (98.75%) | 4.157 | |
| Heterozygous G/A | 3 (5%) | 1 (1.25%) | (0.421-41.005) | | |
| Homozygous A/A | - | - | - | | |
| MTHFR C677T | Allele G | 117 (97.5%) | 159 (99.37%) | 4.076 | 0.226 |
| | Allele A | 3 (2.5%) | 1 (0.63%) | (0.418– 39.690) | |
| | Wild type C/C | 22 (36.66%) | 47 (58.75%) | 2.460 | |
| Heterozygous C/T | 29 (48.34%) | 26 (32.5%) | (1.235 – 4.896) | | |
| Homozygous T/T | 9 (15%) | 7 (8.75%) | - | | |
| PAI-I 4G/5G | Allele C | 73 (60.83%) | 120 (75%) | 1.931 | 0.011 |
| | Allele T | 47 (39.17%) | 40 (25%) | (1.157 – 3.223) | |
| | Wild type 5G/5G | 31 (51.66%) | 50 (62.5%) | 1.559 | 0.199 |
| | Heterozygous 4G/5G | 22 (36.66%) | 28 (35%) | (0.790 – 3.075) | |
| | Homozygous 4G/4G | 7 (11.68%) | 2 (2.5%) | - | |
| PAI-I 4G/5G | Allele 5G | 84 (70%) | 128 (80%) | 1.714 | 0.054 |
| | Allele 4G | 36 (30%) | 32 (20%) | (0.989 – 2.971) | |

*for wild-type vs. heterozygous + homozygous; RPL, recurrent pregnancy loss; OR, odds ratio; CI, confidence interval;

DISCUSSION

Despite the fact that many case-control studies have reported association between RPL and hereditary thrombophilia, the results are heterogeneous and the nature of this association has not been clarified yet (23). In our study we found that factor V Leiden and MTHFR C677T polymorphisms were significantly associated with RPL in Bosnian women, while prothrombin G20210A and PAI-1 4G/5G polymorphisms did not show significant association.

Factor V Leiden is reported as a common inherited risk factor for RPL, with the incidence range of 8-32% in patients and 4-10% in controls (24, 25). Estimated frequencies of FVL allele and genotypes in our research significantly differ between RPL patients and controls. According to the odds ratio (OR), we found that the presence of A allele of FVL was associated with a nearly four times higher risk of recurrent miscarriage in RPL patients. On the other hand in recent study of association between FVL and pregnancy loss in Bosnian women it is not confirmed (26). This discrepancy in the results of those two Bosnian studies could be explained by the fact that in our patients group two women were heterozygous carriers with close relatives with confirmed hereditary thrombophilia caused by FVL mutation. In summarised meta-analyses studies FVL mutation is recognised as significant risk factor for RPL with increased level of risk with $OR \geq 2$ for FVL carriers (2,11). Our results are congruent with those studies where frequencies of FVL were significantly higher in RPL patients than in controls. On the other hand other studies (7) did not find strong association between FVL and RPL.

The findings of this study have demonstrated lower frequencies of prothrombin G2021A polymorphism than FVL, MTHFR C677T and PAI-1 4G/5G polymorphisms. According to the literature, the relative risk for thrombosis is twice higher in individuals with the PT G2021A polymorphism (7). In this study the presence of A allele showed four times the risk for thrombosis event. The frequency of heterozygous carriers of PT G2021A polymorphism reported here was similar to its prevalence in Britain, Italian and Greece RPL patients (27). Alifirevic et al. (28)

reported the same frequency rate of PT G20210A polymorphism in patient and controls. Lack of effect of inherited thrombophilia in women with recurrent pregnancy loss has been reported (29).

Recently, MTHFR C677T polymorphism has been put in focus of investigation of genetics causes of recurrent pregnancy loss (30). In this research we found significant association between MTHFR C677T polymorphism and recurrent pregnancy loss. Similar results were reported by Torabi et al. (31). Several other studies confirmed the role of MTHFR C677T polymorphism in pregnancy complications as preeclampsia, placental infarcts, foetal growth restriction (32, 33). Although, there have been many confirmed results about MTHFR C677T polymorphism influence on recurrent pregnancy loss, its exact role remains unexplained (33).

In a meta-analysis study which included 4306 cases and 3076 controls it is suggested that the PAI-1 4G/5G polymorphism might be associated with recurrent miscarriages in Caucasians (34). On the contrary, in other meta-analyses conducted by Su et al. (35) associations of PAI-1 4G/5G with RPL were not found. Our study did not reveal any correlation of PAI-1 4G/5G polymorphism with the risk of recurrent pregnancy loss.

According to data literature we can conclude that there were contradictions in results among studies of PAI-1 4G/5G associations with RPL. This could be caused by high clinical heterogeneity and difference in sample sizes between various studies.

In conclusion, current results of the studies which confirmed the role of multiple inherited thrombophilic defects in adverse pregnancy outcomes indicate the necessity of prevention of RPL in a way to calculate anticoagulant therapy according to individual expected risk of each patient.

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TRANSPARENCY DECLARATIONS

Competing interests: none to declare.

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