MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPERS

Association of Prothrombin (A20210G) and Factor V Leiden (A506G) with Recurrent Pregnancy Loss

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ABSTRACT

Background: *Inherited thrombophilias are the leading cause of maternal thromboembolism and are associated with an increased risk of recurrent spontaneous abortion (second- and third-trimester fetal loss). The purpose of this study was to investigate the effects of factor V and factor II involved in reproductive failure. Recently a possible association between unexplained infertility and genetic thrombophilia gene mutations have been reported with a significant statistically association with prothrombin A20210G.*

Materials and Methods: *During the period from January 2011 to December 2011, 283 patients with unexplained infertility, who had received in our hospital, were investigated for this retrospective study, and the frequency of polymorphic variations was calculated. The infertile couples with recurrent pregnancy loss (RPL), had been trying to achieve successful pregnancy for greater than 1 year without success and known causes of infertility were excluded (semen anomalies, karyotype abnormalities, uterine malformations, etc) referred to our Centre for genetic counseling. The control group consists of 100 women who had one or more children in history were investigated by DNA Strip.*

Results: *Heterozygous and normal homozygous for the factor V mutation and factor II mutation were equally distributed among patients with recurrent miscarriage and fertile patients with two or more previous births. The combination of the two polymorphisms, prothrombin (A20210G) and factor V Leiden (A506G) revealed a significant correlation between them and early fetal loss.*

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Article received on the 1st June 2012. Article accepted on the 13th August 2012.

Conclusions: *The genes involved in thrombophilia could be one reason for fertility complications in some women with unexplained infertility. Our study shows that there is an association between factor II and V mutation and the risk for fetal loss.*

Keywords: polymorphism, recurrent spontaneous abortions, gene polymorphism, factor V, factor II

INTRODUCTION

Recurrent miscarriage is a pregnancy loss before 20 weeks of gestation (1). The recurrent pregnancy loss usually occurring in the first trimester of gestation and its rate is quite high (15-20% even in full reproductive period) (1,2). In 10-20% of fertile couples, the offspring can be eliminated as an abortion (the first 28 weeks of pregnancy) or a stillborn (after 28 weeks gestation) (2,3). Most miscarriages are due to chromosomal abnormalities, over 50% of early abortions involves removing a product of conception with chromosomal abnormalities (1,4). There are and other conditions which may favor the production of spontaneous abortion such as pelvic infections, diabetes, thyroid disease, thrombophilia.

Thrombophilia was identified as a major cause of recurrent pregnancy loss (RPL), after chromosomal abnormalities with a rate of up to 40%, especially in the first half of pregnancy (5). Because pregnancy is a hypercoagulable state, thromboembolism is the leading cause of antepartum and postpartum maternal mortality. Although numerous studies are available in literature thrombophilia rate seems to vary from study to another due to different selection criteria of patients (6). In this clinical context we can distinguish three types of thrombophilia: inherited thrombophilia, acquired thrombophilia and combined thrombophilia (7,8). Mutations in the gene for factor V Leiden (FVL, G1691A) and prothrombin (FII, G20210A) were considered risk factors for hereditary thrombophilia and can act either in the first half of pregnancy or later in pregnancy, leading to possible miscarriages (9).

Using the most common hereditary thrombophilia, we tested the hypothesis that Factor V Leiden (FVL) mutation (A506G) and Factor II mutation (A20210G) are associated with lower implantation rates. ❑

MATERIALS AND METHODS

During the period from January 2011 to December 2011, 283 Caucasian patients with unexplained infertility, who had received at the Department of Reproductive Medicine, Life Memorial Hospital and Fundeni Clinical Institute of Bucharest Romania, were investigated for this retrospective study, and the frequency of polymorphic variations was calculated. The infertile couples with recurrent pregnancy loss (RPL), had been trying to achieve successful pregnancy for greater than 1 year without success and known causes of infertility were excluded (semen anomalies, karyotype abnormalities, uterine malformations, etc) referred to our Centre for genetic counseling. The control group consists of 100 women all Caucasian, who had one or more children in history with no history of spontaneous abortions, autoimmune disorders or endocrine diseases. Factor V Leiden and factor II mutation were analyzed in genomic DNA extracted from leukocytes of the whole blood using Thrombo Type test based on the DNA Strip (QIAGEN, Hilden, Germany). This technology permits the combined characterization of position 1691 in the factor V gene and position 20210 in the prothrombin gene. The procedure is divided into three steps: DNA extraction from a patient sample (EDTA blood), a multiplex amplification with biotinylated primers and a reverse hybridization. Genomic DNA was isolated from 200–250 uL of buffy coat using the QIAamp Blood Kit (Qiagen). DNA was quantified by measuring the absorbance at 260 nm using a NanoDrop spectrophotometer (ThermoScientific). The amplified segment of DNA was then digested with the restriction enzyme of mutation G1691A of the Factor V gene, G20210A of the prothrombin Factor II gene.

The results of the two groups were compared using the two-tailed Fisher's exact test and calculated online at Graph Pad Software-

website (http://www.graphpad.com/quickcalcs/contingency1.cfm8. ❑

RESULTS

All 283 infertile women and the 100 fertile women of the control group were investigated for the Factor V Leiden and Factor II mutations. Demographic data of the study group are shown in Table 1 in comparison with the controls. No mutant homozygous FVL (A506G) or FII (G20210A) carriers were identified in the control group but in the study group was identified in four cases. Heterozygous and normal homozygous for the FVL mutation and FII mutation were equally distributed among RPL patients and control individuals (Heterozygous for FVL: 7.42% versus 5%, P = 0.64; FII: 2.83% versus 4% P=0.52; Normal homozygous for FVL: 91.87% versus 95%, P = 0.30; FII: 96.47% versus 96% P=0.83; all non-significant) (Table 2).

The allele frequencies and genotype distribution for the FVL (G1691A) and FII (G20210A) polymorphisms does not significantly differ between patients and controls group and there was no significant correlation between these and infertility (data are presented in Table 3 and Table 4).

After correlation between the genotypes of the two polymorphisms FVL (G1691A) and FII (G20210A), was found a statistical association between mutant homozygous for FVL and heterozygous for FII (AA G1691A/GA G20210A) and between heterozygous for FVL and normal homozygous for FII (GA G1691A/GG G20210A). No statistically significant associations were found between other correlations (Table 5). ❑

DISCUSSION

This study was designed to explore the implication of prothrombin A20210G and FVL G1691A in recurrent pregnancy loss. We did not find a strong association a factor V Leiden gene polymorphism and prothrombin A20210G gene polymorphism with recurrent miscarriages, while we did find an increased frequency of association between the two gene polymorphism AA G1691A/GA G20210A and GA G1691A/GG G20210A (Table 5) compared with control group. RPL is classically defined as the loss of three or more consecutive pregnancies before the fetus has reached viability (10,11). Several studies have demonstrated that women with inherited thrombophilias carry a higher risk for recurrent early miscarriages, second-trimester abortion and other complications of pregnancy (12,13). Factor V Leiden mutation might be a significant risk factor with a reported incidence of 8–32% in patients and 4–10% in controls (5,14). Factor V Leiden, is responsible for more than 75% of inherited activated protein C resistance, is the more common inherited thrombotic risk factor associated to RPL (15,16). Ridker et al. has reported an increased prevalence of FVL in women with recurrent pregnancy loss, while other studies found a strong association between FVL and early pregnancy loss (17). Also FVL has been identified as a risk factor also for late RPL (18). On the other hand prothrombin A20210G has been identified as a risk factor for pregnancy

	Study group	Control group
Number of patients	283	100
Age	25-40(33.76%	19-45(32.8%)
Pregnancies	0	1-3
Miscarriages	1-5	0

TABLE 1. Demographic data of recurrent pregnancy loss (RPL) patients and controls

Study group (n=283)	Normal homozygous G/G	Heterozygous G/A	Mutant homozygous A/A
FVL (G1691A)	260(91.87%)	21(7.42%)	2(0.71%)
FII G20210A	273(96.47%)	8(2.83%)	2(0.71%)
Control group (n=100)	**Normal homozygous**	**Heterozygous**	**Mutant homozygous**
FVL (A506G)	95(95%)	5(5%)	0
FII G20210A	96(96%)	4(4%)	0

TABLE 2. Genotype frequencies for polymorphism in recurrent pregnancy loss (RPL) patients and controls.

loss in several studies and has been associated mostly to early RPL (19).

The mutations of the factor V Leiden and prothrombin genes might play a role in implantation failure or in fetal loss after IVF (In vitro fertilisation) (20). The precise mechanism by which thrombophilias affect recurrent pregnancy loss is as yet unknown. It has been suggested that thrombosis of maternal vessels may contribute to the complications in association with thrombophilias (21).

Several studies have reported an association between hereditary thrombophilias and increased complications of pregnancy, such as severe preeclampsia, fetal growth restriction, stillbirth and abruptio placentae (21,20). ❑

CONCLUSIONS

The incidence of factor V Leiden and prothrombin in female patients with fertility problems is not significantly higher than that control group but there was a significant correlation between the combinations of the two polymorphisms. ($P < 0.05$) Pregnancy is associated with an increased risk of venous thromboembolism (VTE), and this condition remains an important cause of maternal morbidity and mortality. Although the available data are limited and flawed, the use of anticoagulation for prevention of adverse pregnancy outcomes in women with heritable thrombophilia is increasing.

Studied groups	Genotype G/G	Genotype A/A	Genotype G/A	G allele frequency	A allele frequency
RPL (n=283)	260	2	21	541	25
%	91.87%	0.70	7.42%	95.58	4.42
Controls (n=100)	95	0	5	195	5
%	95%	0	0.5%		8.13
P	0.86	1	0.64	0.94	0.38

TABLE 3. Allele and genotype frequencies for FVL (G1691A) in recurrent pregnancy loss (RPL) patients and controls.

Studied groups	Genotype G/G	Genotype A/A	Genotype G/A	G allele frequency	A allele frequency
RPL (n=283)	273	2	8	554	12
%	91.87%	0.70%	7.42%	97.88%	2.12%
Controls (n=100)	96	0	4	196	4
%	96%	0	0.4%	98%	2%
P	1	1	0.52	1	1

TABLE 4. Allele and genotype frequencies for FII (G20210A) in recurrent pregnancy loss (RPL) patients and controls.

Genotypes	Study group (RPL)	Control group	OR (odd ratio)	Confidence Interval	P
GG/GG	252	90	0.9032	0.4256 to 1.9165	0.85
GG/AA	2	0	0	0 to infinity	1
GG/GA	7	5	0.4819	0.1494 to 1.5544	0.31
AA/GG	1	0	0	0 to infinity	0.26
AA/AA	0	0	infinity	0 to infinity	1
AA/GA	0	5	0	0	**0.001**
GA/GG	20	0	0	0 to infinity	**0.003**
GA/AA	0	0	infinity	0 to infinity	1
GA/GA	1	0	0	0 to infinity	0.26

TABLE 5. Correlation between FVL (G1691A) and FII (G20210A) factors genotypes in studied group.

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