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ORIGINAL ARTICLE

Study on the Relationship of G20210A Prothrombin mutation with Unexplained recurrent Spontaneous Abortion

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ABSTRACT
Thrombophilia is a multigenic disorder caused by inherited and acquired defects. Pregnancy loss is reported to be more common in women with inherited and acquired thrombophilias. Recurrent fetal loss is a frequent health problem, with three or more and two or more affecting up to 5% of women in the reproductive age. Several specific thrombophilias found to be associated with spontaneous abortions are factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin mutations. The aim of this study was to investigate the relationship between recurrent miscarriages and prothrombin (factor II) blood coagulation factor mutations. We conducted study including a clinically well-defined group of 70 patients with spontaneous abortions to test the association between prothrombin (Factor II) blood coagulation factor mutations and recurrent spontaneous abortions in Iranian population. In the present case control study, blood coagulation factor mutations has been investigated in 65 patients with spontaneous abortions and 80 healthy subjects by using Multiplex PCR and reverse dot blot methods. Then, the data were analyzed by SPSS software. The results of this study did not show considerable association between prothrombin (factor II) blood coagulation factor mutations and recurrent spontaneous abortion in Iranian population.
Keywords: *Thrombophilia, recurrent spontaneous abortion, prothrombin, blood coagulation factor mutations abortion, Multiplex PCR, reverse dot blot method*

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INTRODUCTION

Recurrent spontaneous abortion represents the consecutive loss of at least two pregnancies prior to 20th week of gestation. Thrombophilia is a multigenic disorder caused by inherited and acquired defects. Pregnancy loss is reported to be more common in women with inherited and acquired thrombophilias. After chromosome abnormality, thrombophilia is one of the most important genetic factors that could cause recurrent pregnancy loss. Recurrent fetal loss is a frequent health problem, with three or more affecting 1–2% and two or more affecting up to 5% of women in the reproductive age. [1][2][3]

The most typical and frequent inherited thrombophilias include mutation G1691A in factor V gene (FVL) and mutation G20210A in the 3-UTR of gene prothrombin. Factor V Leiden act by resistance to APC (activated protein C) and G20210A increases plasma prothrombine level. [4][5] Thrombophilia usually causes by point mutation in factor V (G1691A), and factor II prothrombine (G20210A) genes. Factor II prothrombin mutation increases chance of recurrent early pregnancy loss. [6][7]

Inherited thrombophilias contain deficiencies of antithrombin, protein C or protein S; homozygous or heterozygous mutations of factor V (Leiden – G1691A) or prothrombin (G20210A); and homozygosity for the thermolabile variant of methylenetetrahydrofolate reductase. [8]

Specific thrombophilias establish to be related to spontaneous abortions are factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin G20210A mutations, deficiencies of protein C, protein S and antithrombin. [9][10]

The proposed mechanisms of fetal loss in women with thrombophilia include inhibition of the thrombolytic system, thrombosis of placental vessels, placental infarction, and direct cytotoxic effects.(11)There are contentious data on whether prothrombin (G20210A) mutation is more related to first-trimester or with second- trimester miscarriage. The outcome of meta-analysis has suggested that factor II prothrombin carriers have a top threat of late pregnancy loss than first trimester abortion. [12]

Prothrombin could be the inactive precursor of thrombin, which will be required to be able to convert fibrinogen into fibrin, the principal goal of the coagulation cascade. Prothrombin thrombophilia results from an individual base pair substitution of guanine to adenine at position 20210 in the 3'-untranslated region of the prothrombin gene. A prothrombin G20210A defect results in elevated plasma quantities of prothrombin and thrombin, ultimately causing an elevated danger of clot formation. [13][14][15]

In this study a clinically well-defined group of 70 patients with spontaneous abortions and control population contained of 44 age-matched women, with one or more live born children and no history of pregnancy loss. These women were recruited throughout their attendance for pregnancy. All participants were from exactly the same ethnical background, and gave their informed consent before inclusion to the study. As a result, to verify weather inherited thrombophilia may determine the risk of recurrent abortion; we evaluated the prevalence of prothrombin (G20210A) in 70 patients with recurrent abortion and in 80 healthy control women.

MATERIALS AND METHODS

This study is an association study with a case-control design and study population involved of 70 women with recurrent spontaneous abortions, and 80 healthy women matched for age and without previous history of Recurrent Spontaneous Abortions (RSA). Blood samples collection were carried out during the period from June 2012 to October 2013. Blood samples werecollected from each subject. DNA was extracted from whole blood samples and then we used Multiplex PCR and reverse dot blot methods to detect prothrombin (G20210A) mutation in 70 patients with recurrent abortion and in 80 healthy control women. Formerly, the data were analyzed by pasw statistics 18 (SPSS) software.

Patients and controls

DNA was obtained from 70 patients with recurrent spontaneous abortions. A total of 80 healthy controls women without recurrent spontaneous abortions were also analyzed to define a possible role for the prothrombin (G20210A) mutation in Iranian patients in late pregnancy. Genomic DNA was amplified by polymerase chain reaction (PCR) with proper primers.

Multiplex PCR and reverse dot blot methods

Genomic DNA from venous blood samples was isolated using Quick Micro Prep Kit (GeneID, Germany) according to manufacturer's instructions. The prothrombin (G20210A) mutation genotyping was performed base on Multiplex PCR and reverse dot blot methods (GeneID, Germany) the thermal cycling conditions for Multiplex PCR were as(Table1), after that reverse dot blot methodshas been done for detection of prothrombin (G20210A) mutation.

Table1:The thermal cycling conditions for Multiplex PCR

PCR with Fast and Easy extraction solution			
5	Min.	95^{0}C	**1 cycle**
30	Sec.	95^{0}C	**10 cycle**
2	Min.	60^{0}C	**10 cycle**
10	Sec.	95^{0}C	**25 cycle**
30	Sec.	55^{0}C	**25 cycle**
30	Sec.	72^{0}C	**25 cycle**
8	Min.	72^{0}C	**1 cycle**
4^{0}C∞			

Statistical Analysis

Statistical analyses were conducted using with the SPSS software (Statistical Package for Social Sciences) version18. Chi- square test ($\chi 2$), was used to test the association between two categorical variables or to detect difference between two or more proportions. Pearson chi-square was used to investigate the

relationship between recurrent miscarriages and prothrombin (factor II) blood coagulation factor mutations.

RESULTS

We genotyped and analyzed 70 patients with recurrent spontaneous abortions, and 80 healthy controls, for the detection of prothrombin (G20210A) mutations and recurrent spontaneous abortions in Iranian population.

Prothrombin (G20210A) mutations frequencies were in equilibrium in patients and controls. Patients showed an extensively increased frequency of prothrombin (G20210A) mutations compared with controls. Thus the prothrombin (G20210A) mutations would confer a slightly increased risk of developing recurrent spontaneous abortions in Iranian population. Carriers of the prothrombin (G20210A) mutations were at a slightly but not significantly increased frequency in patients compared with controls. Both groups of healthy controls and women withrecurrent spontaneous abortions(RAS), had similar gene frequencies, suggesting that this mutation is not related with recurrent spontaneous abortions in Iranian population (Table2).

Table2:Chi- square test ($\chi 2$) for analyzing the prothrombin (G20210A) mutations

Crosstab				
Count				
		FII		Total
		1	2	
group	case	1	69	70
	cont	0	80	80
Total		1	149	150

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.151[a]	1	.283		
Continuity Correction[b]	.004	1	.947		
Likelihood Ratio	1.532	1	.216		
Fisher's Exact Test				.367	.367
N of Valid Cases	150				

DISCUSSION

Our results showed that the genotype and the allele frequencies of prothrombin (G20210A) mutations were meaningfully different between patients with recurrent spontaneous abortions and the controls (all P-values were 0.467). On the other hand, neither genotype nor allele frequencies of endothelial nitric oxide synthase gene G894T polymorphism were significantly different between RPL patients and the controls (P-values for genotype and allele frequency: 0.367), so, do not seem to contribute to an increased risk for recurrent spontaneous abortions. As a result Lack of association between recurrent spontaneous abortions and prothrombin (G20210A) mutations observed in this study. Contradictory results in associating an allele, genotype and recurrent spontaneous abortions in different populations can be attributed to the variation in the genetic background, in particular linkage disequilibrium to varying genetic elements.

CONCLUSION

This study was conducted in order to determine the association between prothrombin (G20210A) mutations, and recurrent spontaneous abortions. The current study have investigated the relation between prothrombin (G20210A) mutations and the development of spontaneous abortions and showed that there is not significantly association between prothrombin (G20210A) mutations and recurrent spontaneous abortions.

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