

Thrombotic Proteins and Recurrent Abortion

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ABSTRACT

Introduction: Infertility due to recurrent abortion is the common clinical problem in fertility clinics. The research for finding etiology of recurrent abortion, is led to highlight the “thrombotic factors” role in this situation. This work showed the relation between these proteins defect in molecular view in women who suffer from recurrent abortion and consequent infertility. **Materials and methods:** We studied Prothrombin gene G20210A mutation, Factor V Leiden, Fibrinogen, MTHFR mutation and PAI1 in 45 Caucasian infertile women referred to Fertility Center of the private Sarah infertility center and Aragon private medical genetics laboratory, of whom 40 with unexplained, female infertility, and failed to natural pregnancy. **Results:** A significant relationship between inherited thrombophilia MTHFR, Factor V, II, and PAI1 was observed, whereas no association between thrombophilia and early or secondary infertility in female was found. Significantly higher prevalence of MTHFR gene mutation in unexplained infertile women in comparison to that observed in fertile women was observed ($P=0.097$ in early vs $p=0.000$ in secondary infertility); the prevalence of the other thrombophilia determinants was higher, even if not significantly, in the unexplained infertile group. **Discussion:** The detection of complete panel of thrombotic proteins, as their related genes, have the effective role in true diagnosis of recurrent abortion main factor. As the discrepancy is occurred usually in incomplete and selected proteins function in the evaluation of miscarriage cases, the patients must accept the time vesting and more cost and mostly get no accurate results. The studies that performed before in different populations and races, has shown wide range of many kinds of mutations in these proteins and related genes. In this assessment, we evaluated the complete panel of these series in Iranian Fars ethnic group, that mostly referred for repeat the IVF program. As shown data, the most meaningful difference between early infertile women with secondary infertile group, the MTHFR C677T mutation and Factor V, showed highest significant difference in these two groups ($p=0.000$) as the previous studies has reported (11). **Conclusions:** This study demonstrates the relationship between inherited thrombophilia and recurrent abortion and subsequent infertility, that reflects the part of genetic markers in thrombophilia and its influence in women infertility, and explains the reason of IVF procedure failure.

Keywords: Thrombophilia; Proteins; Infertility; Genetic marker

INTRODUCTION

Pregnancy loss in early weeks (less or equal as 10 weeks), is one of the most gynecology problems. From all pregnancies, around 15% is lost and from them almost 1%, return to recurrent abortion. It is established that some of the anti-thrombotic proteins mutation, have deep influence in thrombotic influence that results in miscarriage [1]. The recurrent abortion repeat rate is 24% after 2 losses and 30% after 3 and if exceeds more than 4 pregnancy loss, it is around 40% [2]. Although there are many factors that play a role in etiology of pregnancy lose, but the thrombotic factors, more clearly, thrombophilia stress has a meaningful share in recurrent abortion that some of them are hereditary, and some is classified as acquired type like antiphospholipid antibody syndrome [3,4].

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in thrombophilia panel and both patient and physician must be aware at least about genetic tendency to thrombosis.

METHOD

Study population and design

45 selected infertile women undergoing IVF-ET treatment were enrolled into this study as retrospective design. Only women without male factors or unexplained infertility were entered to sampling. All women, were between 25 and 42 years of age and with the regularly menstruating with two intact ovaries and no previous ovarian surgery also they had healthy and normal uterine cavities reported according the clinical documents signed by clinician. Women with previous history of live birth were excluded. Moreover, women with previous thrombi-embolic phenomena, under anti-thrombotic treatment or with known thrombophilia were excluded from evaluation. The research project was approved by the Sarah Medical Center and an informed consent form was signed by each woman participating in the study. As control, 45 healthy women were selected to voluntarily accompany in the study they had normal ovary function and had at least one child before.

*The thrombophilia panel tests were performed according to manufacturer guidance except to fibrinogen test that designed according to guidelines of NCBI (data not showed).

*PCR performing for amplifying the targeted DNA fragment

*Gel electrophoresis the PCR product

Gel agarose 1.5% is prepared and 4 μ l from each sample and 2-3 μ l loading buffer is used and the samples went to 100 voltage power supply in gel and visualized for the results after confirming the product amplification, they sent to be sequenced according the instructions (ABI prism sequence analyzer).

RESULTS

Among the 45 women entered in the study, Patients' parameters were as follows: Age, infertility duration, order of infertility, as

well as number of previous IVF cycles, were similar between the case and control groups. Parity, number of children and previous abortions were also similar. According to the obtained results, MTHFRA1298C mutations (1298A), the mutation rate in both points were 34.2% compare to 18.4% in non-mutant (wild type) patients (p value: 0.214). The same data is obtained for secondary infertility (p value: 0.367) [Table 1,2]. In MTHFR C677T mutation, there's no significant difference in mutant and wild type patient samples (p value: 0.725) in early infertility and for secondary infertility the p value was about 0.920 in qui-square test (data not shown). The results for other mutations are as follows: PAI1 mutation: (p value: 0.509) in early infertility and in second infertility (p value: 0.504). For FGB mutation in the early infertility was (p value: 0.393) and in secondary infertility was (p value: 0.545), (data not shown) and for factor II (p value :0.684) in early infertility and in second infertility was (p value: 0.743) (F). For factor V or Leiden, the test is failed because of low sample count. All of the tests was performed with two sided qui-square test.

In Spearman Rho relation test between early and secondary infertility comparison the results were as Table 2. The significant difference was showed in MTHFR 677 mutation in early infertile group (p=0.097) and in secondary infertile group (p=0.0000), also in PAI1 gene showed a significant value in secondary infertile group (p=0.067) as in Factor V, both early and secondary infertile groupsb has a very significant values (p=0.000 and p=0.000) respectively, and for factor II, in early infertile group there was a significant differences (p=0.064) corresponding to control groups [Table 2] [Figure 1]. Basal ovarian reserve tests showed clear signs of low ovarian reserve in the study group as compared to the control group. This was manifested by significantly higher serum FSH level, lower AFC and ovarian volume (data not shown). In the early and secondary groups of infertile women, although there is a diversity percent of mutation between homozygote and heterozygote state, but it couldn't find out any meaningful difference in two groups [Figure 2].

Table 1: Comparison of prevalence in thrombotic proteins mutations between heterozygote and homozygote mutants and wild type samples in of A1298C mutation in early infertile patients.

id	(Spearman's rho)Early infertility	(Spearman's rho) Secondary infertility
MTFHR1298	0.261	-0.21
MTFHR677	0.097	0
PAI1	-0.146	0.068
FGB	0.208	-0.168
F2	0.064	-0.51
F5	0	0

Table 2: The comparison between early and secondary infertility and mutations rate difference in thrombotic proteins.

		Early infertility		Total	
		TRUE	FALSE		
MTHFR1298	H	Count	13	0	13
		% within Earlyinfertility	34.20%	0.00%	31.70%
	M	Count	7	0	7
		% within Earlyinfertility	18.40%	0.00%	17.10%
W	Count	18	3	21	
	% within Earlyinfertility	47.40%	100.00%	51.20%	
Total	Count	38	3	41	
	% within Earlyinfertility	100.00%	100.00%	100.00%	

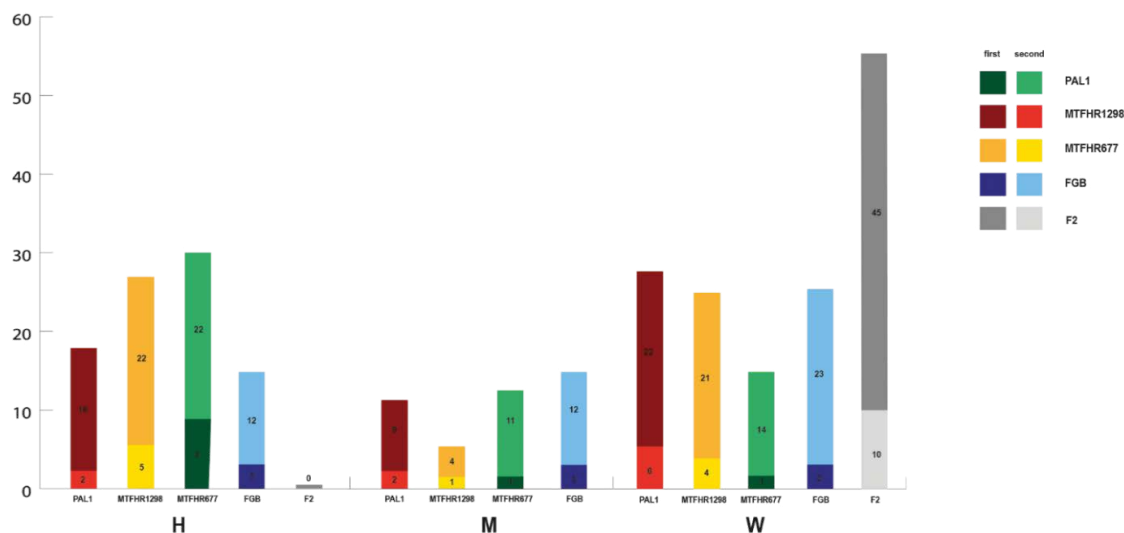


Figure 1: Prevalence of MTHFR A1298C, MTHFR C677T, PAI1, Factor V and Factor II mutations comparison between early and secondary infertility cases.

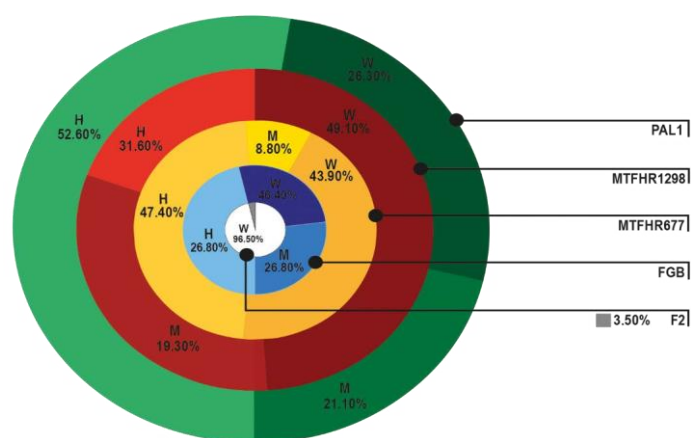


Figure 2: Pie diagram of mutation rate in homo and heterozygote type and wild type (no mutation) of FGB, MTHFR and PAI1 genes in infertile patients.

DISCUSSION

The detection of complete panel of thrombotic proteins, as their related genes, have the effective role in true diagnosis of recurrent abortion mail factor [7,8]. As the discrepancy is occurred usually in incomplete and selected proteins function in the evaluation of miscarriage cases, the patients must accept the time vesting and more cost and mostly get no accurate results. The studies that performed before in different populations and races [9,10], has shown wide range of many kinds of mutations in these proteins and related genes [11]. In this assessment, we evaluated the complete panel of these series in Iranian Fars ethnic group, that mostly referred for repeat the IVF program. As shown data, the most meaningful difference between early infertile women with secondary infertile group, the MTHFRC677T mutation and Factor V, showed highest significant difference in these two groups ($p=0.000$) as the previous studies has reported [12,13].

After MTHFR, the most prevalent mutation in early infertile women was seen in PAI1 gene, ($p=0.068$). As in previous studies, the homozygosity of 4G in PAI-1 and MTHFR C677T genes in women with RPL, and heterozygosity of FVL, FVR2, ACE, and ApoE2 genes, in both parents play crucial role in recurrent abortion and also IVF cycles failure and have strong correlation with a paternal and maternal synergetic effect of fetus loose [14]. Although in qui-square comparison analysis, none of the studied genes and their related proteins showed no significant difference between mutant and wild type samples that might be due to low sample number

that has to be followed in further study. This study demonstrates the relationship between inherited thrombophilia and recurrent abortion and subsequent infertility that reflects the part of genetic markers in thrombophilia and its influence in women infertility, and explains the reason of IVF procedure failure. In aspect of heterozygosity or homozygosity prevalence difference between early and secondary infertile women, it should be noted that the kind of mutated gene's effect for example in this study MTHFR C677 and PAI1 had the most prevalent homozygosity occurrence rate. In addition, beside of careful and complete evaluation of thrombotic genes mutation, it should importantly note about the homozygosity and heterozygosity status of both parents in cases of recurrent abortion and IVF failure.

CONCLUSION

The detection of complete panel of thrombotic proteins, as their related genes, have the effective role in true diagnosis of recurrent abortion mail factor. As the discrepancy is occurred usually in incomplete and selected proteins function in the evaluation of miscarriage cases, the patients must accept the time vesting and more cost and mostly get no accurate results. The studies that performed before in different populations and races, has shown wide range of many kinds of mutations in these proteins and related genes [15]. In this assessment, we evaluated the complete panel of these series in Iranian Fars ethnic group, that mostly referred for repeat the IVF program. As shown data, the most meaningful difference between early infertile women with secondary infertile

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