Factor V H1299r (Hr2) Heterozygosity: A Risk Factor For Recurrent Implantation Failure Particularly In Non-Carriers For Factor V Leiden Mutation-A Case-Control Study

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ABSTRACT

OBJECTIVE: The association between recurrent implantation failure and thrombophilia is still controversial depending on the published reports with conflicting results. In this study, we aimed to assess the clinical relevance of screening women with recurrent implantation failure for some thrombophilic variants including factor V H1299R (FV HR2) haplotype.

STUDY DESIGN: A total of 279 women were recruited in this case-control study. 229 women with a history of recurrent implantation failure and 50 fertile control with no history of pregnancy losses were screened for eight specific gene mutations, regarding factor V G1691A gene (FV Leiden), FV HR2, factor II prothrombin G20210A, factor XIII V34L, PAI-1 4G/5G, MTHFR C677T, MTHFR A1298C and A3 haplotype of the endothelial cell protein C receptor gene.

RESULTS: Recurrent implantation failure group displayed a significantly higher prevalence of FV HR2 heterozygosity than fertile controls while the frequency of FV Leiden mutation was comparable between groups (p=0.011; p=0.619). Additionally, the difference in the prevalence of other specific or total gene mutations among women with recurrent implantation failure was also insignificant.

DISCUSSION: The primer outcome of this study was the co-existence of the higher prevalence of FV HR2 haplotype and the insignificant percentage of FV Leiden mutation in women with recurrent implantation failure. Thus, we emphasize that the HR2 haplotype may be associated with recurrent implantation failure particularly in non-carriers for FV Leiden mutation. In the necessity of screening for thrombophilia in recurrent implantation failure, HR2 haplotype should be involved in the searched gene panel particularly in the absence of FV Leiden mutation. Further large-scale prospective studies are needed to investigate whether screening or treatment for HR2 haplotype has any detrimental impact on implantation success in cases of recurrent implantation failure.

Keywords: Assisted reproductive treatments, Factor V, Recurrent implantation failure, Thrombophilia

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Introduction

Assisted reproductive treatments (ART) are promising techniques in infertility management. However, it may cause a burden for couples struggling with multiple failed attempts who experience the disappointment of a negative serum/urine human chorionic gonadotropin (hCG) level or a biochemical pregnancy in the absence of a visible ultrasonographic intrauterine gestational sac after repeated ART cycles, in summary, a diagnose of implantation failure (1). Several studies have referred to the underlying causes of implantation failure including thrombophilia (2-5). Unfortunately, the contribution of thrombophilia as a susceptible factor in recurrent implantation failure (RIF) is still controversial related to the inconsistent results of previously published reports.

Even though a consensus on a formal definition of RIF is not available, it is usually considered as the absence of achieving a pregnancy after at least three consecutive in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), or

frozen embryo replacement cycles with good quality embryos under age of 40 (6). Implantation involves the attachment of the embryo to the luminal surface of the endometrium which is followed by the migration and invasion through the deeper layers (7). In some reports, the disruption of trophoblastic invasion through local microvascular thrombosis of decidual or chorionic vessels at the site of implantation has been attributed to be the leading factor for implantation failure in thrombophilic variants (8). Mostly, inherited thrombophilia contributes to late fetal loss. But implantation failure is also likely to be associated with thrombophilia through decreased trophoblast invasion of the spiral arteries, endothelial dysfunction and abnormal coagulation activation at the maternal–fetal interface (9). On the contrary, some authors refuse to entitle thrombophilia as a predisposing factor for RIF (7,10).

Eventually, conflicting results regarding the association between thrombophilia and RIF had prompted us to investigate the prevalence of thrombophilic factors including factor V G1691A (FV Leiden), factor V H1299R (FV HR2), factor II prothrombin G20210A, factor XIII V34L, PAI-1 4G/5G, methylenetetrahydrofolate reductase (MTHFR) C677T, (MTHFR) A1298C, and A3 haplotype of the endothelial cell protein C receptor (EPCR) gene in women with a history of RIF.

Material and Method

A total of 279 women were recruited in this case-control study which was conducted in an assisted reproduction unit of a tertiary care center in Turkey. The study was approved by the institutional ethical review board (Ethics approval reference number: E2-21-538). Informed consent was obtained from all patients participating in the study and the study conforms to the provisions of the Declaration of Helsinki. A total of 229 women with a history of RIF and 50 fertile control women with no history of pregnancy losses were screened for specific gene mutations.

The eligibility criteria were considered as a history of negative serum/urine hCG level or a biochemical pregnancy in the absence of a visible ultrasonographic intrauterine gestational sac after the transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles under the age of 40 years, no co-existing medical disorders, and a normal uterine cavity verified by ultrasound or hysteroscopy.

Peripheral blood samples of 2.5 cc were drawn from the cases and put into the standard K3 EDTA tubes. Nucleospin (MN Macherey-Nagel GmbH, Germany)-nucleic acid isolation-kits were used in order to get genomic DNA from peripheral blood samples. After confirmation of presence of isolated DNA by adding to the Agarose gel, amplification was applied.

Peripheral blood was withdrawn and genomic DNA was extracted from samples using standard techniques (11). Thereafter, genotyping was analyzed with strip assay kits (Vienna Lab. Labordiagnostika GmBH, Austria) (12). The products were analyzed for eight specific gene mutations including FV Leiden, FV HR2, factor II prothrombin G20210A, factor XIII V34L, PAI-1 4G/5G, MTHFR C677T, MTHFR A1298C, and A3 haplotype of the EPCR gene. Study and control groups were compared regarding the prevalence of these mutations.

The statistical analyses were performed using SPSS 21.0 for Windows (SPSS, Chicago, IL, USA). Categorical variables are presented as the number of cases and percentages. Pearson's Chi-Square Test was performed for the analysis of categorical variables. A p-value of <0.05 was considered to be significant.

Results

In our results, FV HR2 haplotype had a significantly higher prevalence of heterozygosity in women with RIF than fertile controls (18.6% vs 2%; p=0.011). The heterozygous and homozygous mutations of FV Leiden in RIF were recorded as 10.6 % vs 1.8 % respectively. However, the difference between the study and the control groups did not reach statistical significance (p=0.619). Additionally, the frequency of the other specific or total gene mutations (factor II prothrombin G20210A, factor XIII V34L, PAI-1 4G/5G, MTHFR C677T, MTHFR A1298C and A3 haplotype of EPCR gene) among patients with implantation failure were also similar (p=0.899; p=0.214; p=0.103; p= 0.447; p= 0.777; p=0.839). The prevalence of the mutations is shown in detail in table 1.

Table I: *Prevalence of thrombophilic mutations*

	HOM-IF%	HET-IF%	HOM-N%	HET-N%	*p* value*
Factor V G1691A (FV Leiden)	1.8	10.6	0	12	0.619
Factor V H1299R (FV HR2)	0	18.6	2	2	***0.011***
Factor II prothrombin G20210A	0	4.4	0	4	0.899
Factor XIII V34L	5.7	20	0	24	0.214
PAI-1 4G/5G	23.7	48.2	20	64	0.103
MTHFR C677T	10.6	45.8	12	36	0.447
MTHFR A1298C	10.5	50.4	14	48	0.777
A3 haplotype of EPCR gene	4.3	21.7	2	24	0.839

*HOM: homozygous; HET: heterozygous; IF: implantation failure; N: controls. *Pearson Chi-Square*

Discussion

The association between RIF and thrombophilia is still controversial depending on the published reports with conflicting results. In literature, FV Leiden mutation is a frequently searched susceptible factor in RIF whereas another factor V gene variant, HR2 haplotype has been underestimated (13-15). FV Leiden (1691 G_ A substitution) is one of the most common variants of the FV gene which blocks the inactivation of factor Va by activated protein C (APC) and results in APC resistance. Another nucleotide change in exon 13 of FV gene , HR2 haplotype (4070 A-G transition), is also associated with a mild APC resistance and increased risk of thrombotic events (12). In this study, we found that women with a history of RIF had displayed a significantly higher prevalence of HR2 heterozygosity than fertile controls while the frequency of FV Leiden mutation was comparable between groups.

It has been postulated that the HR2 haplotype is solely associated with an increased risk of venous thromboembolism (VTE) in the absence of FV Leiden mutation with an odds ratio of 2.0 (95% CI: 1.2-3.5, p=0.01) (16). Moreover, co-inheritance of factor V Leiden and HR2 heterozygosity leads to a 3- to 4-fold increase in the relative risk of VTE with a severe APC resistance phenotype in comparison with factor V Leiden alone (17). In light of these, the tendency for thrombotic events in presence of H2 haplotype of factor V gene may also contribute to implantation failure through local microvascular thrombosis of decidual or chorionic vessels at the site of implantation.

In literature, the number of studies searching the association between implantation failure and HR2 haplotype has been limited. In a prospective cohort study, 1717 patients undergoing their first fresh ART cycle with no exclusion criteria were screened for several thrombophilic mutations, and no significant differences in embryo implantation were reported including HR2 haplotype (18). In a study including 42 women with a history of recurrent implantation failure, Coulam et al did not observe any association between RIF and HR2 haplotype (19). Further, in some studies the presence of HR2 haplotype was found to be associated with the risk of recurrent pregnancy loss (RPL): Goodman et al assessed that HR2 haplotype solely was not correlated with RPL risk, however in total analysis with other mutations, the association reached to a statistical significance (10). In another study, it was suggested that heterozygosity of HR2 haplotype in both parents (20) should be considered as a risk factor for RPL.

Zaher et al concluded that in VTE patients, non-carriers of FV Leiden mutation were 2,7 times more likely to have HR2 haplotype as compared to controls (p=0.036, 95% CI=1.04-7.06) (21). In our study of implantation failure, we obtained similar results supporting the coexistence of the higher prevalence of HR2 haplotype and the insignificant percentage of FV Leiden mutation in women with RIF. Thus we emphasize that the HR2 haplotype may be associated with RIF particularly in non-carriers for FV Leiden mutation.

The limitation of the study was that the concurrent status of FV Leiden and FV HR2 mutations were not defined in groups separately. Thus, the frequency of FV Leiden−/FV HR2+ patients could not be calculated by itself in the RIF group.

Routine screening for thrombophilia in RIF is still a matter of debate. In a review by Ata et al, it was concluded that testing or treatment for congenital thrombophilia in RIF should be recommended in patients with a personal or family history of VTE (22). If screening is required, HR2 haplotype should be involved in the searched thrombophilic gene panel especially in the absence of FV Leiden mutation.

Due to high prevalence of MTHFR polymorphisms in fertile couples, anticoagulant prophylaxis is not required and supplementation with folic acid is just recommended. But the presence of a possible association between FV HR2 and RIF still remains elusive, so the role of anticoagulation with low dose aspirin or low molecular weight heparin (LMWH) in such cases warrants further evaluation. Large-scale studies are needed to determine whether screening or treatment for HR2 haplotype in the factor V gene has any detrimental impact on implantation success in RIF.

Declarations

Acknowledgment Presented as a poster presentation at 69th Conjoint Meeting of the International Federation of Fertility Societies and the American Society for Reproductive Medicine Boston, October 12-17, 2013.

Ethics approval and consent to participate: All participants signed informed written consent before being enrolled in the study. The study was reviewed and approved by the ethics committee of the University of Health Sciences, Ankara City Hospital

Availability of data and materials: The datasets and code used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions: Constructing the hypothesis or idea of research and/or article ASOE; Planning methodology to reach the conclusions ASOE; Organizing, supervising the course of progress and taking the responsibility of the research/study NY, SE; Taking responsibility in patient follow-up, collection of relevant biological materials, data management, and reporting, execution of the experiments ZC, SY; Taking responsibility in logical interpretation and conclusion of the results ASOE, PGC; Taking responsibility in necessary literature review for the study PGC; Taking responsibility in the writing of the whole or important parts of the study PGC; Reviewing the article before submission scientifically besides spelling and

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