AMHSR ORIGINAL ARTICLE

Association of estrogen receptor 1 rs9340799 polymorphism with implantation failure in Iranian infertile women

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ABSTRACT

Purpose: Estrogen as a crucial hormone during pregnancy acts by two types of receptors. Estrogen receptor alpha, expressed by estrogen receptor 1 (ESR1) gene, is more abundant and exists in all human reproductive systems. Association of ESR1 gene polymorphism has been shown in some reproductive fields such as spontaneous abortion, endometriosis-related infertility and in vitro fertilization failure. The present study has investigated the association between single nucleotide polymorphism rs9320799 in intron 1 of ESR1 gene with implantation failure in Iranian infertile women who were submitted for conventional in vitro fertilization (IVF) procedure and had no blastocyst implantation.

Materials and Methods: Hundred two infertile women with at least one IVF failure were enrolled in the study as the case group and 112 healthy women as the control group. The restriction fragment length of polymorphism method was performed for genotyping the single nucleotide polymorphism rs9340799 (A/G XbaI) located in intron 1 of ESR1 gene. The result was confirmed by DNA sequencing analysis.

Results: The genotype distribution of AA in case and control groups was 40 (39.2%) and 55 (49.1%), respectively. However, genotype distribution of AG was 41 (40.2%) and 47 (41.9%). Ultimately genotype distribution of GG was 21 (20.6%) and 10 (9%). There was a statistically significant difference between case and control groups in the genotype distribution of XbaI polymorphism ($P = .04$).

Conclusion: There was an association between single nucleotide polymorphism ESR1 rs9340799 and implantation failure in Iranian infertile women who underwent IVF procedure.

Keywords: implantation failure; estrogen receptor 1 gene; single nucleotide polymorphism; infertility; genotype distribution.

AMHSR 2014;12:116-120
www.journals.ajaums.ac.ir

INTRODUCTION

During implantation process, a receptive uterus is an essential factor that guaranties success of implantation and pregnancy.[1,2] Factors, involved in the process, prepare the uterine luminal epithelium to its best situation for blastocyst reception that finally makes a cross-link between uterus and blastocyst. Here, estrogen hormone plays a crucial role throughout the entire pregnancy, from regulating the production of progesterone to fetal development.[3] This important role of estrogen has been proven in the uterine preparation for the implantation in mice.[4]

Estrogen acts through its nuclear receptors which have two subtypes in human, i.e. ER-α and ER-β coded by ESR1 and ESR2 genes on 6q25 and 14q23.2 loci, respectively.[5] ER-α, the most abundant, is found in all human reproductive tissues. Studies on ER-α knockout mouse have revealed its role in reproduction.[6] Single

nucleotide polymorphism rs9340799 is located in intron 1 of ESR1 that has a restriction site for XbaI (A/G) enzyme. Some association studies have revealed that this single nucleotide polymorphism can modify susceptibility to some reproductive disorders such as endometriosis, spontaneous abortion, osteoporosis, controlled ovarian hyperstimulation and idiopathic secondary premature ovarian failure.[7-10] Recently, a report elucidated that ER-α gene polymorphism is associated with endometriosis-related infertility and in vitro fertilization (IVF) failure in Brazilian women.[11]

This study has investigated the association of polymorphism XbaI in ESR1 gene with unsuccessful blastocyst implantation in Iranian women who had undergone conventional IVF.

MATERIALS AND METHODS

A total of 102 women, less than 40 years old, who underwent an initial intracytoplasmic sperm injection (ICSI) procedure with oocyte retrieval and embryo transfer, entered the study as the case group. Their ethnic origin consisted of different ethnics of Iran and none of the participants had any relationship with each other. Before the ICSI procedure, the patients had been evaluated in terms of their personal and family medical and reproduction history. Participants with potential cause of infertility such as endometriosis, thrombophilias and thyroid disease were excluded from the study. All participants had at least one IVF failure cycle and were chosen from an Iran's Army Air Force hospital. The control group consisted of 112 healthy fertile women who already had two or more children. All participants were informed about the procedures of the study before participation and signed a consent form. The study protocol was approved by ethics committee of Besat hospital.

A blood sample (5 mL) was taken from each patient and genomic DNA was extracted using the high salt extraction method by Miller and colleagues.[12] Polymerase change reaction (PCR) followed by restriction fragment length polymorphism was used as genotyping method. A PCR fragment of 346 bp was amplified by the following primers:

XbaI forward primer, 5'-GATATCCAGGGTTAT-GTGGCA-3' and XbaI reverse primer, 5'-AGGTGTT-GCCTATTATATTAACCTTGA-3'.[13]

The PCR was carried out in a total volume of 50 µL. Reaction mixtures consisted of 100 ng template DNA, 50 ng of the forward primer, 50 ng of the reverse primer, 200 µM each dNTP, 10 mM Tris-Hcl (pH = 8.3), 50 mM KCl, 3 mM MgCl2, and 1 unit Taq DNA polymerase. A denaturation step was first performed (5 min at 94ºC) followed by 35 PCR cycles (denaturation: 45 sec at 94ºC; annealing: 45 sec at 53ºC; extension: 45 sec at 72ºC) and then a final extension phase (7 min at 72ºC). After PCR, the amplified product of 346 bp was digested overnight at 37ºC with XbaI restriction enzyme (Fermentas, Maryland, USA). The digested products were detected on 3% agarose gel electrophoresis. Two fragments of 148 and 198 bp were observed in the presence of XbaI restriction site in which *A* allele is present. DNA sequencing was performed on 10% of the PCR samples to confirm the PCR followed by restriction fragment length polymorphism results.

Genotype distributions were examined for significant departure from Hardy–Weinberg equilibrium by χ^2 test. A χ^2 test was also used for analyzing genotype distributions and frequencies of alleles. The Statistical Package for Social Sciences (SPSS) software version 21 was used for all of the statistical analysis. A *P* value less than .05 was considered to be statistically significant.

RESULTS

Totally, 102 infertile patients with at least one failure in embryo transfer in conventional IVF (case group) and 112 women in fertile group who had at least two children without assisted reproduction (control group) were studied. The quality of extracted DNA is shown in Figure 1. Treatment of XbaI enzyme in three different

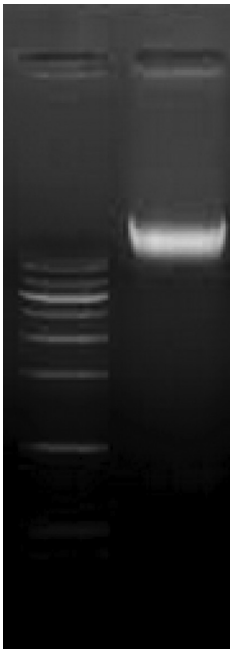

Figure 1. Agarose gel electrophoresis of the DNA extracted from the blood sample of the infertile studied women.

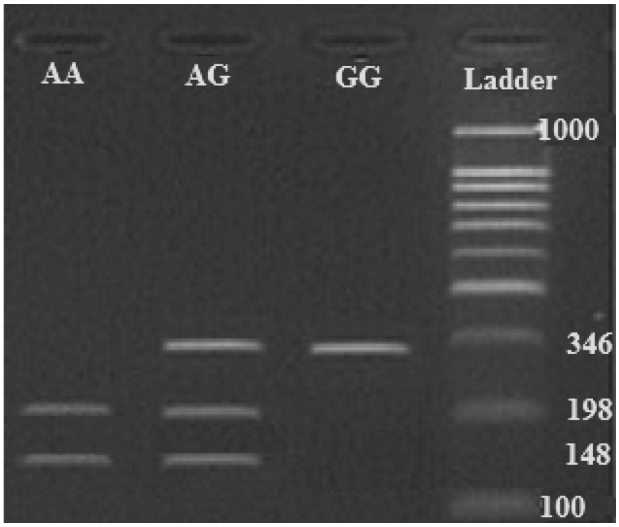

Figure 2. Agarose gel electrophoresis pattern of three different genotypes treated by XbaI restriction enzyme.

genotypes (AA, AG and GG) is shown in Figure 2. As noted before, XbaI treatment resulted in two 148 and 198 segments in which allele A is present in the genotype, then the genotype AG produced three different segment (digested and undigested) because of presentation of both alleles.

The genotype distributions of XbaI polymorphism in the present population in healthy and infertile groups were in the Hardy-Weinberg equilibrium with the P values of 1 and 0.091, respectively. The frequencies of XbaI genotypes in the control group were 55 (49.1%), 47 (41.9%), and 10 (9%) for AA, AG, and GG, respectively. The genotype frequencies were 40 (39.2%), 41 (40.2%), and 21 (20.6%) for AA, AG, and GG in infertile group, respectively. Allele frequencies of A and G alleles in the control group were 0.7 and 0.3 and in the infertile group they were 0.59 and 0.41, respectively. An association was found between the ESR1 rs9340799 polymorphism and clinical phenotype in infertile group ($P = 0.04$) compared to the control group (Table 1). Table 2 shows the allele frequency in both groups.

Table 1. Genotypes frequency of estrogen receptor 1 (ESR1) rs9340799 polymorphism among fertile group and infertile patients.*

Genotype	Fertile	IVF Failure	*P* Value
AA	55 (49.1)	40 (39.2)	.04
AG	47 (41.9)	41 (40.2)	
GG	10 (9)	21 (20.6)	

Key: IVF, in vitro fertilization.
*Data are presented as no (%).

Table 2. Frequency of allele A and G in fertile and IVF failure group.

Allele	Fertile	IVF Failure	*P* Value
A	0.7	0.59	.1
G	0.3	0.41	

Key: IVF, in vitro fertilization.

DISCUSSION

Even in the best assisted reproductive technology units, pregnancy rate is not more than 30%.[14] Different factors may be involved in failed pregnancy during the cycle, such as inappropriate ovarian stimulation, unsuitable uterus milieu and suboptimal laboratory culture conditions.[15]

Implantation failure is the most common cause of lack of pregnancy after embryo transfer in IVF procedure. It takes place in about of 40% of IVF experiments.[16] Some evidences indicate that genetic factors regulate implantation process. Thus, genetic defects and polymorphisms influence increasing susceptibility to process failure both directly and indirectly.[17,18] Among the factors involved in the pregnancy, estrogen hormone has a crucial role. Fetal growth and development during both extra- and intra-uterine periods of life depends on estrogen produced by placenta.[19] As it was mentioned before, null female mice with knockout ESR1 gene are infertile, with no corpus luteum formation and altered gonadotropin levels.[20]

Considering the pivotal role of estrogen and its receptors during pregnancy, some studies have been done to find out whether there is any association between ESR variants and factors involved in pregnancy. Single nucleotide polymorphism rs9340799 is located in intron 1 of ESR1 gene with no amino acid changes, but it is possible that it can influence ESR1 gene expression and subsequently pregnancy fate.

Paskulin and colleagues demonstrated a significant association between ESR1 rs9340799 polymorphism and women with endometriosis-related infertility and those who had no blastocyst implantation in Brazilian population.[11] Anousha and colleagues also showed that there is a meaningful association between XbaI and PvuII genotype in intron 1 of ESR1 gene and the decreased risk of spontaneous abortion.[7] Also, it has been revealed that ESR polymorphisms have association with ovarian response to follicle stimulating hormone in a person who undergoes IVF procedure.[9] Based on an investigation in Turkish infertile women regarding IVF parameters such as numbers of collected oocyte, maturation and embryo quality, it has been suggested that these parameters could be associated with estrogen receptors variants.[21]

In this study we tried to find an association between XbaI variant in ESR1 gene and blastocyst implantation failure in women who undergo conventional IVF cycles. Our results showed that there is some association though not significant between the two. This is an agreement with results of a similar study in which single nucleotide polymorphism rs9340799 had a significant association (P = .018) with implantation failure among infertile Brazilian women. Effect of ESR1 gene variants over expression level of ESR1 gene may be useful to clear the potential role of these variations, but the exact mechanism behind the association remains unclear so far.[22]

Considering the high medical cost and mental burden of couples who suffer from infertility, doing genetics screening along with clinical screening seems necessary before using artificial techniques. Beside other genomic markers, high valuable single nucleotide polymorphisms that have a significant association with infertility can be promising to avoid unpleasant results from failure cycles.

CONCLUSION

There seems to be an association between XbaI (A/G) polymorphism in ESR1 gene and infertile women who had implantation failure during IVF cycles in Iranian women. For future studies, array-based single nucleotide polymorphism genotyping can help to indicate more significant association or potential point mutation in genes that had not been known as genes involved in infertility.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

The authors would like to thank the personnel of IVF and infertility treatment clinic at Besat hospital for their assistance.

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Received July 2014
Accepted August 2014