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Original Research Article

Association of FSH receptor promoter's polymorphisms with IVF-failure in Iranian women

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ABSTRACT

Background: Follicle-Stimulating Hormone Receptor (FSHR) gene shows five Single Nucleotide Polymorphisms (SNPs) in the promoter region at positions -29, -37, - 114, -123 and -138 that have been reported to be associated with higher levels of FSH and various ovarian responses to FSH in IVF (*In-vitro* fertilization) treatment at different populations. Hence, they are important regulators of hormone activity at the target level in IVF process. This study was performed to investigate the association between FSHR gene polymorphisms and IVF failure in Iranian women.
Methods: SNPs in the promoter region of FSHR gene were analyzed by PCR and direct sequencing technique in 90 women in three equally sized groups of IVF failure, IVF success and normal fertile women, using genomic DNA extracted from white blood cells.
Results: No significant differences were found in allelic variants frequency and genotype distribution between each category of subjects when analyzing the FSHR SNPs in the promoter region (p-value >0.05). However, analysis of the data revealed that the subjects with A/A genotype at the –29 position received higher amount of exogenous FSH for ovulation induction compared to G/G genotypes.
Conclusions: These results indicate that the FSHR SNP at position –29 may influence sensitivity of the FSHR to FSH for ovulation induction in IVF treatment. It may be concluded that the A/A genotype at position –29 is associated with poor ovarian response to FSH so that subjects with A/A genotype at the –29 position may require higher doses of exogenous FSH for ovulation induction during IVF process.

Keywords: FSHR gene promoter, Infertility, IVF failure, *In-vitro* fertilization

INTRODUCTION

Infertility is an inability to become clinically pregnant after a period of 12 months or more of regular sexual intercourse.[1] With a worldwide incidence of 12-15 percent, infertility is a difficult challenge faced by young couples. In Iran, infertility rate is estimated to be approximately 20-21 percent.[2] Infertility results from a combination of both genetic and environmental factors. Study of the exact causes of infertility in couples leads to achieve effective way of treatment procedures. In Vitro

Fertilization (IVF) is a costly method of assisted reproductive technology (ART) used to help over 80 million couples worldwide which involves fertilization of an egg with sperm in a laboratory dish and transferring fertilized eggs into the uterus. IVF failure causes significant emotional and financial burdens on infertile couples. Therefore, in this procedure finding the causes of IVF failure and formulating solutions for them is very important. Follicle Stimulating Hormone (FSH), a glycoprotein secreted by the anterior pituitary gland, has a significant role in the ovulation process by stimulating the ovaries, regulating estradiol hormone secretion and maintaining pregnancy.[3] Follicle Stimulating Hormone Receptor (FSHR) gene is one of the factors associated with infertility and IVF failure. In both males and females, the interaction between FSH and its receptor, FSHR, is essential for normal spermatogenesis and oogenesis.[4] As FSH plays a central role in stimulating follicular growth, it is used for controlled ovarian stimulation during IVF protocols and for various other infertility treatments. However, efficient doses of exogenous FSH for ovarian stimulation varies between women in different populations; leading to the suggestion of predictive factors for ovarian response. Therefore, determining the dose of FSH to attain optimum ovarian response is an ongoing challenge in the field of infertility management in IVF clinics. Common stimulation protocols are used in IVF but the ovarian response to exogenous FSH widely varies ranging from poor to hyper responses. Many parameters, such as age and diminished ovarian reserve have been used as a marker to predict ovarian response to FSH. FSHR gene, the most studied gene in infertility, encodes a protein which belongs to the family of G-protein-coupled receptors, expresses exclusively in ovarian granulose cells in women, and is involved in FSH signal transduction via the cAMP pathway. More than 1300 SNPs in the promoter and coding regions of the FSHR gene have been identified worldwide with studies focusing on their association with ovarian response in the IVF process.[5] Recently the association of a SNP at position -29 in the 5'-untranslated regions of FSHR (g. -29G>A) (rs1394205) has been delineated with poor ovarian response and reduced FSHR expression. The human FSHR promoter is lacking a conventional TATA and CCAAT box with multiple transcriptional start sites, and has a transcription factor binding site for the viral E26 transformation specific sequence (cETS-1) which is the location of rs1394205 SNP.[6,7] Due to differences in response to FSH in various populations, studying polymorphisms in this region among Iranian women is necessary. This study examined the FSH receptor promoter's polymorphisms and their association with infertility and IVF failure. For this purpose, blood samples were obtained from30 women with naturally conceived pregnancies, 30 infertile women with unsuccessful IVF and 30 infertile women with successful IVF. Polymorphisms of the promoter region of FSHR gene was assessed using PCR and direct sequencing. The results demonstrated no significant differences in allelic variant frequencies and genotype distribution between each category of subjects and controls when analyzing the FSHR SNPs in the core promoter region (p-value >0.05). However, analysis of the data revealed that the subjects with A/A genotypes at the –29 position received higher amount of exogenous FSH for ovulation induction when compared with G/G genotype subjects. These results indicate that SNP of the core promoter region at position–29 of FSHR gene may influence sensitivity of the FSHR to FSH in vivo, and it may be concluded that the A/A genotype at position –29 associated with the poor ovarian response to FSH, so that, women with A/A genotype at the –29 position required higher amount of exogenous FSH for ovulation induction during IVF process.

METHODS

Samples

This cross sectional, case control study was performed on patients who were referred to infertility clinic at Dr Shariati hospital, Tehran, Iran. After obtaining informed consent from all participants, blood samples were obtained from 30 women with naturally conceived pregnancies, 30 infertile women following successful IVF and 30 infertile women with unsuccessful IVF. The procedures followed were in accordance with the ethical standards of Tehran University of Medical Sciences and the Helsinki Declaration of 1975, as revised in 1983.

DNA extraction and PCR sequencing

From each subject, 5 ml of blood was drawn with EDTA added as anticoagulant. White Blood Cells (WBCs) was isolated from the whole peripheral blood. Genomic DNA was extracted from WBCs using the Phenol - Chloroform method.

Gene Runner software and Oligo Expree1.4 were used to design primers for promoter region of FSHR gene. The specificity of the primers was checked using the NCBI/ primer-BLAST. Primer sequences designed as following: FSHR-forward primer: 5'-TCGGGAGGTCAGAAGGAA-3' and FSHR-reverse primer´: 5'-GAATGGTGAACAGCAAGCAGA-3' which amplifies a 444bp product size. Polymorphisms at FSHR gene promoter region were identified by PCR Sequencing. Each PCR reaction contained: 1µL DNA, 1µl forward primer, 1µl reverse primer, 2µL DMSO 10% and 12µl Master Mix. Aliquot was made up to a final volume of 25µL by adding8µl distilled water. Amplification was performed under the following conditions: first denaturation at 95 °C for 5 minutes followed by 30 cycles of second denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds and extension at 72°C for 30 seconds, and final extension at 72 °C for 5 minutes. To confirm the specific amplification, all PCR products were electrophoresed on the 2.5% agarose gel. PCR products were sequenced by ABI-3130 automated sequencer.

Statistical analysis

Data processing and statistical analysis were performed under Statistical Package for the Social Sciences, (SPSS) version 23. These tests include Chi-square test, to compare many variables that are active in various levels, and Mann Whitney test, to compare mean scores, in pairs, associated with project topics.

RESULTS

To identify SNPs in the promoter region of the human FSHR gene, genomic DNA was extracted from WBCs. The promoter fragment was amplified by PCR technique using genomic DNA. The 444 bp DNA fragment was attained by agarose gel electrophoresis (Figure 1).

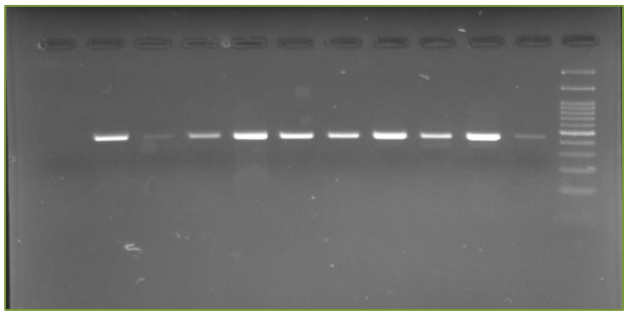

Figure 1: Electrophoresis of the PCR product of the FSHR promoter region.

PCR-sequencing analysis in the core promoter (–1 to –231) yielded 5 SNPs at positions: –138 (A→T), –123 (A→G), –114 (T→C), –37 (A→G), and –29 (G→A). The highest frequency of polymorphism was found at position –29, whereas the other four incidences of polymorphism were found in lower frequencies. The FSHR sequencing result for position -29 is illustrated in Figure 2 (a, b and c).

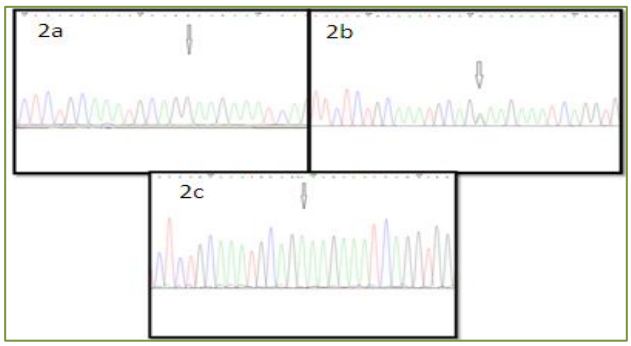

Figure 2: The graph of the FSHR sequencing result at position -29: a) homozygous G/G genotype, b) heterozygous A/G genotype, c) homozygous A/A genotype.

In the three categories of subjects, the extension detection of polymorphisms at -29 were as follows: 13.3% for A/A, 46.6% for A/G and 40.1% for G/G in IVF-failure group and13.3% for A/A, 43.3% for A/G and 43.4% for G/G genotypes in IVF success group and 6.6% for A/A, 26.7% for A/G and 66.7% for G/G in normal pregnancy group (Figure 3).

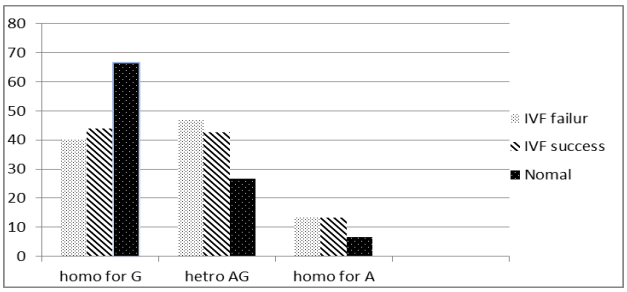

Figure 3: Distribution of the single-nucleotide polymorphism at position -29 in IVF failure, IVF success and normal women.

At position -37 the genotype distribution for IVF failure women included 97.8% for homozygous A/A, 2.2% for heterozygous A/G and 0% for homozygous G/G and for IVF success and normal pregnancy women all samples were homozygous for A/A genotype.

At position –114 in IVF-success women 95.6% were homozygote T/T, 4.4% were heterozygote T/C and none of the samples were found to be homozygote C/C. Two other groups of samples (normal and IVF failure) had T/T genotype in all samples.

The distribution of genotype A/A for both positions –123 and -138 were 100% in all three categories of IVF failure, IVF success and normal women. Comparing the hormone doses in two groups of IVF success and IVF failure with the U Mann – Whitney test demonstrated a significant difference between the dose of FSH administered in IVF success group compared to IVF failure (p value <0.05, 95% confidence level-Figure 4a, b).

In other words, those with A/A at position -29 received much more FSH for ovulation induction compared with G/G genotype (Figure 4a, b).

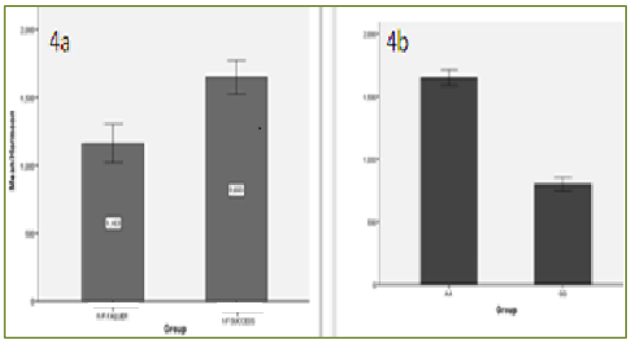

Figure 4: Comparing the FSH hormone therapy a) Comparing successful IVF and IVF failure women. b) Comparing IVF- failure women having A/A genotype with G/G genotype.

DISCUSSION

The incidence of infertility is estimated to be 12-15% worldwide and is more frequent in Iran at about 20-21%.[3] Various genetic and environmental factors are known to have a role in infertility. Among them, FSHR promoter's polymorphism is one of the associated factors with infertility which to the best of our knowledge has been studied in most populations except in Iran. The present study examined the FSHR promoter polymorphisms and their association with infertility and IVF failure. The results demonstrated no significant differences in allelic variants frequency and genotype distribution between each category of subjects when analyzing the FSHR SNPs in the core promoter region (p-value >0.05). However, subjects with A/A genotypes at the –29 position compared to G/G genotype subjects, received higher doses of exogenous FSH for ovarian stimulation and ovulation. The normal FSHR gene function is essential for successful fertility in women based on studies done on FSHR knockout mice. It has been shown that FSHR knockout mice were infertile and their phenotype was similar to the one observed in infertile women with an inactivating mutation in FSHR.[8] In addition, the polymorphisms in the promoter and coding region of FSHR gene have been studied in association to ovarian responses in women (g._29G>A, p.Thr307Ala and p.Asn680Ser).[8] Although some studies suggested the coding region polymorphisms as a potential predictor marker for ovarian response, others demonstrated no significant association.[9-13]

FSHR core promoter region shows five single nucleotide polymorphisms, at positions -29, -37, - 114, -123 and -138, that have been reported to be associated with higher level of FSH and various ovarian responses to FSH in IVF at different populations. The present study analyzed the association of polymorphisms at promoter of FSHR gene with ovarian response to FSH stimulation in Iranian women. The results demonstrated no significant difference in allelic variants frequency and genotype distribution between each category of subjects in the core promoter region (p-value >0.05). However, this study indicated the association of the allelic of promoter polymorphism genotypes at positions -29 (rs1394205) with ovarian response to FSH stimulation in Iranian women. This result has been alongside with the study that has been done in vitro in CHO cell line which showed the correlation of A allele at position -29 with lower expression of FSHR gene in comparison with G.[14] The loss of cETS-1 transcription factor binding site might be the cause of these findings. On the other hand, another study indicated the association of reduced FSHR expression with poor ovarian response in women undergoing IVF.[15]

Analysis of the data delineated subjects with A/A genotype at position -29 received higher dose of exogenous FSH for ovulation induction, in comparison with G/G genotype subjects. The results indicate that SNP of the core promoter region at position -29 of FSHR gene may influence sensitivity of the FSHR to FSH in vivo, and that the A/A genotype at position -29 associated with poor ovarian response to FSH, so that subjects with A/A genotype at the -29 positions require higher doses of exogenous FSH for ovulation induction during IVF process. The findings of a study carried out in Germany are similar to our results, indicating no association between promoter polymorphisms in FSHR gene and ovarian response. Another study compared women with A/A genotype at -29 position of FSHR gene with G/G genotype and revealed that the women with A/A genotype require higher dose of exogenous FSH together with lower amount of estradiol, lower number of follicles and oocytes which is reflected in our findings.[16] Exogenous FSH is administrated to women undergoing IVF and variable ovarian responses have been reported, ranging from high response to the minimal dose of FSH which causes ovarian hyper stimulation syndrome (OHSS), to poor response even with higher doses of FSH which leads to low retrieved mature oocytes.[17] The poor responders may need to repeat stimulation cycles with higher financial burden. According to the present results which indicated that SNP of the core promoter region at position –29 of the FSHR gene may influence sensitivity of the FSHR to FSH in vivo, it may be suggested that women with A/A genotype at position –29 will show poor ovarian response, and so will require higher doses of exogenous FSH for ovulation induction during IVF process.

Controversial findings in different populations of the effect of FSHR polymorphisms on infertility, demonstrated the need for more investigation at a molecular level and also in other populations. However, to confirm the present results, a larger number of Iranian infertile women with successful and unsuccessful IVF should be examined. Thus, the additive effect of such genes must be studied to uncover possible genetic causes of infertility in detail and formulate personalized treatments for infertile women.

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