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Original Research

Association of leukemia inhibitory factor gene polymorphism and in vitro fertilization outcome in a population in northern Iran

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Abstract: Several studies have been demonstrated that endometrial leukemia inhibitory factor (LIF) is important in embryo implantation. LIF is a secreted glycoprotein with a variety of biological functions including stimulation of cell proliferation, differentiation and survival that are all essential for blastocyete development and implantation. The LIF receptor activates several signaling pathways in diverse cell types, including Jak/STAT, MAPK and PI3-kinase pathways in the endometrium of fertile woman. It has been suggested that the initial lower expression of LIF in proliferative phase may be one of the causes for multiple failure of implantation. The aim of this study was to evaluate the association between maternal genotype of SNP 3951C/T LIF and in vitro fertilization and embryo transfer (IVF-ET) outcome in infertile women. This case–control study was comprised of infertile patients (n=70) and women having one healthy child as controls (n=73). Genotyping for SNP-3951C/T was performed by PCR/RFLP. Allele and genotype distribution did not differ significantly between patients and controls (P>0.05). The LIF genotype frequencies amongst the 70 cases were C/C=40%, C/T=52.8% and T/T=7.2%; the C and T allele frequencies were 66% and 34%, respectively. The LIF genotype frequencies amongst the 73 controls were C/C=45.20%, C/T=50.70% and T/T=4.1%; the C and T allele frequencies were 70% and 30%, respectively. In conclusion, the results of this study indicate that SNP 3951C/T of LIF may not be associated with IVF-ET outcome in this population. Although more studies should be considered with larger number of patients and control subjects to confirm our results.

Key words: Embryo implantation; In vitro fertilization; Embryo trnansfer; Leukemia inhibitory factor; Genetic polymorphism.

Introduction

Infertility is a disorder of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Fertility requires the production of a viable oocyte, transportation through the fallopian tube, and fertilization by a viable spermatozoan. The resulting zygote must then enter the uterus and get implanted into a suitably prepared endometrial lining (1). A defect in any of these steps will result in infertility. In vitro fertilization and embryo transfer (IVF-ET) is the most successful of the infertility treatments and for many people is the last possibility of pregnancy (2). Despite progress in assisted reproductive technology (ART), including IVF-ET, people undertaking IVF often face considerable risk of failure. There are many possible cause that may be responsible for this failure, including inappropriate stimulation protocol, a genetic abnormality in the gametes, and embryo implantation (3). Implantation is a crucial step for establishing pregnancy, requires molecular and cellular events resulting in uterine growth and differentiation, blastocyste adhesion, invasion and placental formation. Successful implantation of the blastocyst into the endometrium is essential for reproduction. Implantation is a complex process which requires synchronization of events in the developing embryo and receptive endometrium, and involves factors such as immune cells, cytokines, growth factors and cell adhesion molecules (4).

Several studies have suggested that endometrial leukemia inhibitory factor (LIF) is important in embryo implantation (5-7). LIF is a secreted glycoprotein and a highly glycosylated 40-50 KDa glycoprotein with a range of biological functions (8), including stimulation of cell proliferation, differentiation and survival that are all essential for blastocyete development and implantation (9). The pleiotropic effects of LIF are accomplished by binding to heterodimeric LIF receptor (LIFR), which consists of two transmembrane proteins, LIFR and gp130. The LIF receptor activates several signaling pathways in diverse cell types, including Jak/STAT, MAPK and PI3-kinase pathways in the endometrium of fertile woman, LIF protein and mRNA are expressed throughout the menstrual cycle with a striking increase in the mid- and late- secretary phase and in early pregnancy (10, 11). Strong expression of LIF mRNA has also been detected in human decidual leukocytes, which are abundant at the implantation site, suggesting that LIF may mediate interactions between maternal decidual leukocytes and invading cytotrophoblasts (12). LIF may contribute to the development of ectopic pregnancies and that pharmacologically targeting LIF-mediated trophoblast outgrowth was shown to be useful as a treatment for ectopic pregnancy (13). It has been suggested that the initial lower expression of LIF in proliferative phase may be one of the causes for multiple failure of implantation (14).

LIF protein has been detected in human uterine flushings during the time of expected implantation in fer-

tile women. Interestingly, the concentration of LIF in the flushings from women with unexplained infertility was significantly lower than those obtained from fertile women at the same time (15). It has been demonstrated that mutations in the LIF gene decrease the biological activity of LIF in the endometrium and cause an implantation failure (16). Taken together, it is suggested that both the pre-implantation embryo and the uterus are sites of LIF action. In this study the 3951 C/T polymorphic site in LIF exon 3 which is located in the 3' UTR has been investigated in relation to IVF-ET outcome. This polymorphism has been shown to reduce mRNA stability and may thus have an effect on the amount of secreted LIF (17). The aim of this study was to investigate the possible association between LIF gene polymorphism and the IVF-ET outcome in a population in northern Iran.

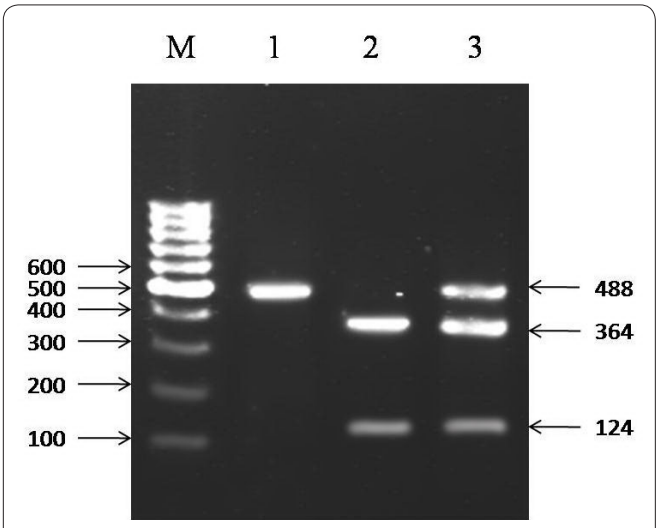

Figure 1. Detection of the LIF 3951C/T by PCR-RFLP. M=100-bp DNA ladder, Lane 1 = T/T; lane 2= C/C; lane 3=C/T.

Materials and Methods

Clinical samples

In this case–control study, 70 infertile patients enrolled who had unsuccessful IVF history and 73 control subjects who had at least one child were randomly selected among individuals visiting hospitals for regular health checks. Patients were recruited from Alzahra Hospital, IVF section, Rasht, Iran. All these subjects were women with median age of 34±6.2 years. A structured questionnaire was used during an in-person interview to elicit information on demographic features. Further, maternal pathology or genetic anomaly, maternal inflammatory disease, uterine malformation, diabetes, lupus erythematosus and embryonic aneuploidy were excluded from the study. The blood samples were collected from both groups. This study has been approved by the local ethical committee and that consent has been obtained from patients.

Polymerase chain reaction-restriction fragment length polymorphism (PCR– RFLP) analysis

Genomic DNA was extracted from EDTA anticoagulated whole peripheral blood using DNG plus (Cinnagen, Iran). To analyze the 3951C/T polymorphism, we amplified a region of the 3′- UTR of LIF gene with forward primer 5′-AGGGGCAGGTTGCTAAGTCAG-3′ and reverse primer 5′-CCCCATTCTCTCAGATCCGA-3′ (18) (BIONEER, Republic of Korea). The primers were designed by means of Oligo7 software (version 7.54, USA). PCR reactions were performed in 25µl reaction volume containing 1µl of each primer (100pmol/µl), 2.5 µl of 10x reaction buffer (100 mM Tris–HCl pH 8.3 at 25°C, 500 mM KCl, 15 mM $MgCl_2$), 0.5 µl of dNTPs (2.5 mM), 0.75 µl of $MgCl_2$, 0.3 µl of Taq DNA polymerase (Cinnagen, Iran), 4 µl of genomic DNA (80 ng/µl) and 14.95 µl H_2O. PCR cycle conditions consisted of an initial denaturation step of 95ºC for 5 minutes followed by 35 cycles of 30 seconds at 95ºC, 30 s at 57ºC, 1 min at 72ºC, and a final extension at 72ºC for 10 minutes. The PCR product fragments were digested with StuI restriction enzyme (Fermentase, USA) for 4.5 hours at 37ºC. Digested fragments were separated by electrophoresis on 2% agarose gels for 40 minutes at 70 V.

Statistical analysis

In order to assess a possible distortion in allele frequencies between cases and controls, we performed a chi-square test with one degree of freedom for both allelic and genotypic distributions between the groups of cases and controls. Significant association was defined by $P \leq 0.05$.

Results

LIF 3951C/T polymorphism was analyzed in all the samples (Figure 1). Tables 1 and 2 show the allelic and genotypic distributions of the polymorphism in cases and controls. We compared the distribution of LIF 3951C/T polymorphism, using χ^2 test.

There were no statistically significant differences in the allelic frequencies or genotype distributions between cases and controls (P>0.05). The LIF genotype frequencies amongst the 73 controls were C/C=45.2%, C/T=50.70% and T/T=4.10%; the C and T allele frequencies were 0.70% and 0.30, respectively. The LIF genotype frequencies amongst the 70 cases were C/C=40%, C/T=52.80% and T/T=7.2%; and the C and T allele frequencies were 0.66% and 0.34%, respectively.

Discussion

Implantation failure is a major reason of infertility in otherwise healthy women. Genetic reason is suspec-

Table 1. Genotype frequencies for LIF C3951T in infertile women undergoing IVF treatments and control groups.

SNP	Genotype	Control group (n =73)	infertile women undergoing IVF (n =70)
C3951T	CC	45.2%(33)	40%(28)
	CT	50.7%(37)	52.8%(37)
	TT	4.1%(3)	7.2%(5)

Table 2. Allelic frequencies in infertile women undergoing IVF treatments and control groups.

Allele frequency(%)	C	T
Control group	70	30
Infertile women undergoing IVF	66	34

ted, but the underlying gene alterations are not understood. Both the embryo and the endometrium produce factors that may contribute to successful implantation (19). LIF was shown to be important in the implantation process (7). LIF is a multifunctional glycoprotein which has an important role in reproduction. LIF is expressed in human endometrium in a menstrual cycle manner. Maximal LIF expression was observed on days 19-25 of the menstrual cycle coinciding with time of blastocyste implantation (20). It has been demonstrated that women with recurrent implantation failure (RIF) have significant lower LIF protein levels in the endometrial glandular endothelium compared with controls (6).

It has been shown that heterozygocity for LIF gene mutation leading either to decreased specific biological activity of the LIF protein that may be a cause of either failure or decreased efficacy of implantation and thus be responsible for infertility in a subgroup of nulligravid women (16). Potentially functional mutations in the LIF gene do occur in women with unexplained infertility and play a role in the etiology of infertility. Genetic polymorphism has been shown to be important in IVF-ET outcome. A specific TP53 haplotypes was shown to be associated with an increased risk of post-IVF failure (21). It was also shown that VEGF-1154 A/A gene serve as a susceptibility factor affecting the chances of recurrent implantation failure (22). It has been demonstrated that progesterone gene receptor, P53, estrogen receptor, MMP9 and GSTM1 gene polymorphisms are associated with the risk of IVF-ET outcome in infertile women (23, 24-28).

No significant change in the frequencies of the genotypes was seen between infertile patients compared with controls. Thus the data of this study indicate that LIF genotype is not associated with the outcome of IVF in infertile women in this population. Further study in other populations will verify whether it is associated with outcome of IVF in infertile women or not. Infertility is multifactorial in origin and other genetic and environmental factors may be contributing to the infertility phenotype in the studied population.

In conclusion, the results of this study indicate that SNP 3951C/T of LIF may not be associated with IVF-ET outcome in this population. Further studies are needed to confirm the role of LIF gene in IVF-ET outcome.

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