Original Article

Association between the Genetic Variation of LIF R Gene with Its Gene Expression and Implantation Outcomes of IVF-Iraqi Arab Females

Dr. Zainab Sabah Hassab[1], Dr. Asmaa M. Salih Almohaidi[2], Dr. Fadhila S. Alsaidi[3]

[1]Lecturer, Osol Aldeen University College, Iraq

[2]Professor, College of Science for Women, University of Baghdad, Iraq

[3]Gynecologist, Private Djla Hospital/ Ministry of Health, Iraq

Abstract

Background: Infertility causes an important social and psychological stressor amongst couples everywhere, particularly among Iraqi women. It is a couple's failure to conceive following one year at least of unprotected intercourse. Only 15% of in vitro grown embryos are implanted successfully. LIF R genes variants are a new crucial bio-indicator for predicting the success of IVF implantation amongst infertility females. LIF R gene is participates in the blastocyst implantation into the endometrium. Also, the LIF R gene act as an anti-inflammatory function, when tissue inflammation. Therefore, LIF R gene expression increased during implantation.

Study Aims: The present study aims to determine effect gene expression and genetic variants of the leukemia inhibitory factor receptor (LIF R) gene in the implantation outcomes in female Iraqi Arab infertility patients.

Materials and Methods: Samples were collected from Kamal Al-Samarrai hospital due to the period from December 2019 to November 2020. The samples were split based on IVF implantation outcomes.

Results:. At first time: the current study recorded a new SNP 5557, (C>G) of the LIF R gene among the success implantation group only. While, rs1012350134 SNP 65661, (A>G) was affected (P<0.001) through modify GG as a risk factor in failed IVF implantation 72.7% than 33.3% in successful implantation. AG frequency has statistically important 9.1% for failure comparison 58.3% in success implantation. Reduced gene expression of LIF R genes is caused by polymorphism in the LIF R genes.

Conclusion: The genetic variance can be screening method for endometrial receptivity, to IVF -implantation improving, or susceptibility risk of failure implantation

Keywords: LIF R, Genetic Variation, IVF, Implantation outcomes.

INTRODUCTION

Infertility is a global health issue worrying of the couple's life (1). It performed prevalence around (9-18) % in overall population (2). Endometrial dysfunction may be detected as one of the important causes of infertility (3).

Address for correspondence: Zainab Sabah Hassab
Lecturer, Osol Aldeen University College, Iraq
Email: Zainab@ouc.edu.iq

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.04.230

How to cite this article: Zainab S H, Asmaa M. S A, Fadhila S. A, Association between the Genetic Variation of LIF R Gene with Its Gene Expression and Implantation Outcomes of IVF-Iraqi Arab Females, J PHARM NEGATIVE RESULTS 2022;13(4): 1665-1671.

The infertile couples undergoing medical help by the drugs, but many of them are unable in having a child. Therefore, they preferred treatment by in vitro fertilization system (IVF) (4). Only 25–30% of the embryos implanted successfully clinically in infertile females after exogenous hormone stimulation (5). Implantation is the primary barrier to the success of both IVF and natural cycles (6).

Quality of the embryos, endometrial receptiveness, and cooperation factors such as: cytokines, growth factors, and hormones are all important for successful implantation (7) (8). The cytokine dysfunction is more severe in patients with RIF (9). LIF receptor protein has high affinity with its cytokine (LIF) by participation the gp130 transduction . The LIF R located on chromosome 5 (5p13.1). It has a linear DNA and 127443 bp long. It includes 24 intron and 25 exons (10). The endometrium and blastocysts expressed mRNA of LIF R. This indicates the role of LIF R in embryo_ maternal interaction during implantation (11). LIF R is localized on the luminal epithelial cells to stimulate the endometrium by autocrine action. Also, it stimulated the blastocyst attachment by LIF- paracrine action, which a cytokine essential for blastocyst implantation (12).

The clinical implantation can be linked to increased or decreased expression of the genes that encode for proteins in the endometrium according to (13) (14). Additionally, LIF receptor has another function as an anti-inflammatory action. Therefore, LIF R gene expression increased, since the tissue inflammation occurred during implantation (15). LIF R mutant impaired the placentation and lower the nutrients. The later leads to fetal bone volume reduction and reducing the astrocytes in spinal cord (16). Another study suggests that LIF R protein can lead to a chain of events in uterus, which allows a successful implantation. (17).

The current study aimed to investigate the role of this gene (LIF R) in implantation outcome of IVF-Iraqi Arab females, across diagnosis its variation and gene expression.

Materials and Methods:

The present study was focused on the Iraqi Arab infertile women, who had undergo an IVF program (intracytoplasmic sperm injection ICSI) after taking ethical consideration from participations. The samples were obtained from the Kamal Al-Samarrai hospital at embryo transfer (ET). Samples collection were due to period between December 2019 and November 2020. The patients have an age mean 29 years and range of 19 – 45 years. The studied samples were divided into two groups according to the IVF implantation outcomes:

- Implantation Failure Group (50 infertile Women)
- Implantation Success Group (24 infertile Women)

The blood samples (5ml) were collected into an EDTA tube to extract DNA and RNA. The DNA and RNA extracted according to the protocol Taiwanese Geneaid - company of two groups studied (success and failure implantation). cDNA done according to the Korean Bioneer company protocol by using random primer. The DNA and cDNA were stored at -20˚C. DNA primers sequences designed by present study for two regions of the LIF R gene (ID: 3977): exon 1+ intron (extended from 5001-6101 bp) and exon 2+ intron (extended from 64740-65840 bp). Primers were supplied by Bioneer Company, as a lyophilized product. LIF R primers sequences are summarized as the following:

Gene	Regions	Primer Sequence	GC%	Product Size(bp)
LIF R	Exon 1	Forward Primer AGAACGTGTCTCTGCTGCAA	50.0	684
		Reverse Primer CAGGCACTCTAAAAGTCTTCATGTT	40.0	
	Exon 2	Forward Primer GACTTGAGGAGGGTGCAGAT	55.0	740
		Reverse Primer AGTGGAGGCCATACACAAGG	55.0	

LIF R _PCR program (DNA) consisted: Initial denaturation 94 ˚C, 5min at 1 cycle then, 40 cycles for each of: denaturation 94 ˚C for 30sec. , annealing 61 ˚C for30 sec., extension 72 ˚C for3 0 sec. and final extention 72 o C for 5min. . PCR products (24 samples) were sent for check of LIF R variation by using ABI3730XL, automated DNA sequence. Macrogen–Korea. The results were analyzed by using Genious software.

cDNA program performed due to 35 cycles: primer annealing 37ºC, 10min., cDNA synthesis 42ºC, 60min. and heat inactivation 95ºC, 5min. The fold change was detected by using of LIF R gene primer, designed by the present study and the human reference gene β-actin, designed by Macrogen Company. The primers sequencing are summarized as the following:

LIF R gene (Target gene)		Product Size(bp)
Forward	CCTTGGGCAGGAAGGATACTG	196
Reverse	GCCCCTCTGCCTAGCATAAAA	
***B-Actin* gene (Reference gene)**		
Forward	GGGCGGCACCACCATGTACC	90
Reverse	GACGATGGAGGGGCCCGACT	

Threshold cycle (Ct) recorded by done relative RT-qPCR technique program (initial denaturation 95ºC, 8min(1cycle), followed (50 cycle) for each of denaturation 95ºC, 15 sec and annealing 60ºC, 30 sec.). Also, the melting temperature was done to certain the optimal temperature (60 ºC) of primers annealing.

The Results:

The current study involved the two regions of LIF R to determine the association between this gene and IVF implantation outcomes (success and failure implantation).

This study revealed two genetic variances:

- Non Registered SNP, 5557 (C>G) -Transversion for 1st region - LIF R

Also, the current study was revealed to SNP, 5557 (C>G) -Transversion for 1st region - LIF R. This 5557 (C>G) SNP was recorded for first time in the current study.

The statistical analysis showed that the sample is not agreement with HWE (P<0.05), at X2 = 9.197 for the success implantation group. This SNP does not record among Iraqi infertility females, who the failed IVF implantation group (Table 1).

Table (1): Genotypes Observed and Expected Frequencies (Exon 1+ Intron Region) Not Registered SNP, C>G -5557 for LIF R by Using HWE*.

***LIF-R* Gene Not Registered SNP, C>G-5557**						
Genotypes Groups		**CC**	**CG**	**GG**	**Chi-square X^2**	**P-Value**
Success Implantation	**Observed No.**	**20**	**2**	**2**	**9.197**	**0.002****
	Expected No.	**18.4**	**5.3**	**0.4**		

**significant (P<0.01).

The reference genotype frequency CC (100%) was seen in failure implantation females during IVF. While, three genotypes (CC 83.3%, CG 8.3%, and GG 8.3%) are seen in success implantation females. The results do not record significant P>0.05 with non-registered (C>G)- 5557 SNP genotypes of LIF R gene and IVF implantation outcomes at OR values = 10.7561, 0.1837, and 0.1837 at receptively.

The C allele has an equivalent frequency 84.6% in the success implantation females. Thus, this allele frequency hasn't any significant differences (P>0.05) between success and failure implantation.. The current study not recorded the mutant G allele in failure IVF implantation. Therefore, the current study cannot from the comparison detection of the mutant G allele between the two studied groups.

Model selection of Non- Registered SNP, (C>G) of LIF R Gene: There are non- significant differences under recessive and dominant models in genotypes frequency of both CC+CG, GG and GG+ CG, CC among success and failure implantation subjects during IVF (OR =5.4444 and 0.0930 at receptively, P>0.05).

Table (2): Genotyping, Alleles Frequency and Model selection of Non- Registered SNP, C>G -5557 of LIF R and IVF Implantation Outcome

LIF-R Gene Not Registered SNP, 5557 (C>G)						
Genotypes and Alleles Frequency		Study Groups		Odds Ratio	CI 95%	P-value
		Success Implantation No. (%)	Failure Implantation No. (%)			
CC		20(83.3%)	24(100%)	10.7561	0.5463 to 211.7884	0.1182NS
CG		2(8.3%)	0	0.1837	0.0084 to 4.0361	0.2824NS
GG		2(8.3)	0	0.1837	0.0084 to 4.0361	0.2824NS
C		22(84.6%)	24(100%)	9.8000	0.4991 to 192.4240	0.1330NS
G		6(15.4%)	0	Not detected	Not detected	…….
Dominant Model	GG+ CG CC	4(16.7%) 20(83.3%)	0 24(100%)	0.0930	0.0047 to 1.8306	0.1182NS
Recessive Model	CC+CG GG	22(91.7%) 2(8.3%)	24(100%) 0	5.4444	0.2478 to 119.6390	0.2824NS

NS non-significant P>0.05

• rs1012350134 SNP , (A>G)- Transition 65661 for 2nd region- LIF R:

The current study shown the rs1012350134 SNP, (A>G) 65661) of exon 2+ intron region for LIF- R polymorphism.

The results was agreement with HWE for rs1012350134 SNP, (A>G) in 2nd region- LIF R. Non- significant difference between the observed and expected frequencies of genotypes for the success implantation group according to the Chi-square (X2 = 1.344). While, this rs1012350134 SNP, (A>G) has perversion highly significant (P<0.001) of HWE in the failure implantation (X2 = 12.085) (Table3).

Table (3): Genotypes Observed and Expected Frequencies (Exon 2+ Intron Region) rs1012350134 SNP, (A>G) 65661 for of LIF-R by Using H WE.

LIF-R Gene rs1012350134 SNP, (G>A) 65661						
Groups \ Genotypes		AA	AG	GG	Chi-square X^2	P-Value
Success Implantation	Observed No.	2	14	8	1.344	0.23NS
	Expected No.	3.4	11.3	9.4		
Failure implantation	Observed No.	4	2	16	12.085	0.001***
	Expected No.	1.1	7.7	13.1		

***significant (P<0.001), NS non-significant (P>0.05).* Some samples were lost as the result as the purity or transfer conditions of samples

The frequency of AA genotype presented no significant differences among failure and success implantation groups during IVF, (18.2% vs 8.3%), respectively, odd ratio (OR = 2.4444, P>0.05). The AG genotype frequency 58.3% in success implantation compared with the failure implantation 9.1% has statistically significant, when OR = 0.0714, P<0.001). In additional, the mutant GG genotype frequency revealed a highly statistical significance among failure implantation 72.7% than 33.3% in success implantation at OR 5.3333 and CI95%=1.5050 to 18.8998, P< 0.001).

For allele frequency: In spite of the OR value 10.4630, the reference A allele do not have effect (P>0.05) in incidence of implantation (A=21.7% vs 37.5%). Also, modifying G allele does not have an effect in the incidence of IVF implantation (OR=2.16, G=78.3% vs 62.5%) in failure and success implantation at receptively.

Model selection of rs1012350134 SNP, (A>G) of LIF R Gene: There are non-significant differences under a dominant model in genotypes frequency percentage of both combined GG+AG and AA genotypes among success and failure implantation subjects during IVF, (OR = 0.4091and P>0.05). There were significant differences under a recessive model in frequency percentage of both combined AA+AG 66.7% and GG 27.3% genotypes among success and failure implantation subjects during IVF (OR = 0.1875, P< 0.05).

Table (4): Genotyping and Alleles of rs1012350134 SNP , (A>G) 65661 of LIF R and IVF Implantation Outcome of Iraqi Infertility Women.

***LIF R* Gene rs1012350134 SNP, (A>G) 65661**						
Genotypes and Alleles Frequency		**Study Groups**		**Odd Ratio**	**CI 95%**	**P-Value**
		Success Implantation No. (%)	**Failure Implantation No. (%)**			
AA		**2(8.3%)**	**4(18.2%)**	**2.4444**	**0.4008 to 14.9084**	**0.969NS**
AG		**14(58.3%)**	**2(9.1%)**	**0.0714**	**0.0135 to 0.3775**	**0.0019*****
GG		**8(33.3%)**	**16(72.7%)**	**5.3333**	**1.5050 to 18.8998**	**0.0095****
A		**18(37.5%)**	**10(21.7%)**	**0.4630**	**0.1859 to 1.1528**	**0.0980NS**
G		**30(62.5%)**	**34(78.3%)**	**2.16**	**0.86 to 5.52**	**0.095NS**
Dominant Model	**GG+AG**	**22(91.7%)**	**18(81.8%)**	**0.4091**	**0.0671 to 2.4950**	**0.3326NS**
	AA	**2(8.3%)**	**4(18.2%)**			
Recessive Model	**AA+AG**	**16(66.7%)**	**6(27.3%)**	**0.1875**	**0.0529 to 0.6644**	**0.0095****
	GG	**8(33.3%)**	**16(72.7%)**			

significant (P<0.01)*significant (P<0.001), NS non-significant(P>0.05), Some samples were lost as the result of the purity or transfer conditions of samples

LIF R Gene Expression: Table (3) showed the Ct reading mean of the LIF R gene (28.95±0.81) of Iraqi IVF implantation failure. While; the Ct reading means was (26.41±0.44) of success IVF implantation group. Also, the B-actin gene of Ct reading means for failure 23.67±0.46 and 22.82±0.33 for the success IVF implantation. In addition, ΔCt means of failure and success of IVF implantation were 5.29±0.36 vs. 3.59±0.27. The ΔΔCt calculation means of failure and successful IVF-implantation were 1.7±0.36 vs. 0.01±0.27. Thus, the fold

change (2-ΔCt) results of Iraqi infertility females were 0.59±0.12 amongst failure of IVF-implantation than 1.23±0.13 amongst the success group. This means the gene expression of the LIF R gene decreases to around half amongst failure of IVF-implantation than the success group.

Table (5): LIF R Gene Expression of IVF Failure vs. Success Implantation at Embryo Transfer (ET) Time.

Group	Ct of *LIF R* Gene Mean±ES	Ct of *B-actin* Gene Mean±ES	ΔCt Mean±ES	ΔΔCt Mean±ES	Fold Change of *LIF R* Mean±ES	P Value
Failure implantation	**28.95±0.81**	**23.67±0.46**	**5.29±0.36**	**1.7±0.36**	**0.59±0.12**	**0.001****
Success implantation	**26.41±0.44**	**22.82±0.33**	**3.59±0.27**	**0.01±0.27**	**1.23±0.13**	

***significant (P<0.001).

Statistical analysis: It done by using the statistical package of the social sciences (SPSS)_ version 20, H.W.E calculator Online and WINPEPI software _version 11.65.

Discussion

The current design refer to the effect non-registered SNP, (C>G) -5557 at the first time and rs1012350134 SNP, (A>G) 65661 of LIF R gene in implantation of Iraqi infertility women during IVF. The reference genotype CC has a frequency (100%) among failure implantation females. This result can belong to the internal marriage amongst Iraqi IVF infertility females. Therefore, the present study cannot estimate the balance of HWE law among failure implantation women. There are non-registered (C>G) SNP among success implantation . Also there is highly Significant of rs1012350134A>G SNPamong failure implantation. These a perversions can be due to natural selection amongst the Iraqi population study.

Non-registered SNP, (C>G) -5557 recorded among success implantation only. Thus, this design suggests the number increasing can influence the clinical role of this SNP in the IVF implantation outcomes. Also, the current study revealed that model selection to non- registered SNP of LIF R is undergoing to Co-dominant trait of two alleles. Therefore, the current study refer to frequency lower of G allele, that do not recorded among failure IVF implantation group. This study showed the model of selection less appearance probability as a recessive genetic trait.

The present study refers to the important heterozygous AG genotype among the success IVF implantation group. Also, the current results showed the effect of the mutant genotype GG as a risk factor in failed IVF implantation among Iraqi IVF-infertility females. However, it can be referred to non-significant association of mutant allele A in success IVF implantation.

LIF R proteins are present in the human endometrium with a maximum level during implantation. LIF R is expressed by blastocyst and human endometrium. These facts supported the role of the endometrial LIF in the success of blastocyst implantation by binding its receptor (18). There are limited information around gene expression of LIR R gene in human (19).

Current results supported the (13) study through reducing gene expression of LIF R among secondary infertility females. Other designs revealed to LIF R expression was significantly reduced in the epithelial cells of infertile than fertile females (20). Another result showed does not assessed statistical differences for LIF R mRNA amongst infertility females (21). Other studies were distributed LIF R expression to impaired STAT3 activation, as a direct cause for recurrent implantation failure (22) (23).

The current study noted that reducing of LIF R gene expression lead to inhibitor of (LIF–LIF R) complex action by compete with LIF R surface proteins (desensitization).

Conclusion

In general, LIF R gene has effect on IVF implantation outcome through success or failure implantation. Therefore, the current results come as support proof to LIF R gene role in initial attachment of embryo and occurrence of implantation.

References

1 Tasnim N, Begum R. Study on thyroid hormone status in case of female infertility. Anwer Khan Mod Med Coll J. 2017;8(2):117–120.

2 Hanson B, Johnstone E, Dorais J, Silver B, Peterson CM, Hotaling J. Female infertility, infertility-associated diagnoses, and comorbidities: a review. J Assist Reprod Genet. 2017;34(2):167–

177
3 Razdan PN, Chanceller V. Practical management of ovulation induction .(2016). J B Medical Ltd, India.
4 Bergart, MN. The experiance of women in unseccessful infertility treatment . J S work in H care. 2000; 30(4): 45-69.
5 Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update. 2006;12(6):731–746.
6 Steck T, Giess R, Suetterlin MW, Bolland M, Wiest S, Poehls UG, et al. Leukaemia inhibitory factor (LIF) gene mutations in women with unexplained infertility and recurrent failure of implantation after IVF and embryo transfer. Eur J Obstet Gynecol Reprod Biol. 2004; 112(1):69–73.
7 Kim S-M, Kim J-S. A Review of Mechanisms of Implantation. Dev Reprod. 2017; 21(4):351–9.
8 Aghajanova L. Leukemia inhibitory factor and human embryo implantation. Ann N Y Acad Sci. 2004;1034:176–183.
9 Kim S-M, Kim J-S. A Review of Mechanisms of Implantation. Dev Reprod. 2017; 21(4):351–359.
10 Sallam HN. Embryo transfer: Factors involved in optimizing the success. Curr Opin Obstet Gynecol. 2005; 17(3):289–298.
11 Hambartsoumian E. Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. Am J Reprod Immunol. 1998; 39(2):137–143
12 Tawfeek MA, Eid MA, Hasan AZ, Mostafa M, El-Serogy H. Assessment of leukemia inhibitory factor and glycoprotein 130 expression in endometrium and uterine flushing: a possible diagnostic tool for impaired fertility. ABMC Women's Health.2012; 12(1):1-6.
13 Sharkey AM, Smith SK. The endometrium as a cause of implantation failure. Obstet. Gynaecol. 2003;17(2):289–307.
14 Moberg C, Bourlev V, Ilyasova N, Olovsson M. Endometrial expression of LIF and its receptor and peritoneal fluid levels of IL-1α and IL-6 in women with endometriosis are associated with the probability of pregnancy. Arch Gynecol Obstet. 2015; 292(2):429–437.
15 Heydarzadeh, MJ, Mehmannava, Y.Effects of rs929271 SNP in Leukemia Inhibitory Factor Gene on Recurrent Pregnancy Loss, Placental Location and Fetal Gender. Gene Cell Tissue. 2019; 6(2):1-10.
16 Davis SM, Pennypacker KR. The role of the leukemia inhibitory factor receptor in neuroprotective signaling. Pharmacol Ther. 2018; 183:50–57.
17 Kimber SJ. Leukemia inhibitory factor in implantation and uterine biology. Reprod. 2005;130:131–145.
18 Ware CB, Horowitz MC, Renshaw BR, Hunt JS, Liggitt D, Koblar SA, et al. Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. Development. 1995;121(5):1283–1299.
19 Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proc Natl Acad Sci U S A. 1996; 93(7):3115–3120.
20 Siarkou C, Prapas Y, Petousis S, Milias S, Ravanos K, Kalogiannidis I, et al. LIF and LIF-R expression in the endometrium of fertile and infertile women: A prospective observational case-control study. Mol Med Rep. 2016;13(6):4721–4728.
21 Mikolajczyk M, Wirstlein P, Skrzypczak J. Leukaemia inhibitory factor and interleukin 11 levels in uterine flushings of infertile patients with endometriosis. Hum Reprod. 2006;21(12):3054–3058.
22 Subramani E, Madogwe E, Ray CD, Dutta SK, Chakravarty B, Bordignon V, et al. Dysregulated leukemia inhibitory factor and its receptor regulated signal transducers and activators of transcription 3 pathway: A possible cause for repeated implantation failure in women with dormant genital tuberculosis? Fertil Steril. 2016;105(4):1076-1084.
23 Aghajanova L, Altmäe S, Bjuresten K, Hovatta O, Landgren BM, Stavreus-Evers A. Disturbances in the LIF pathway in the endometrium among women with unexplained infertility. Fertil Steril. 2009;91(6):2602–2610.