

E Selectin Gene Polymorphism in Female under InVitro Fertilization Program

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Abstract

Selectins play a role in human implantation. This study focus on the role of SELE, especially the rs5368 and rs3917458 polymorphism genes, has been determined in implantation with expression level. The study involved 100 women undergone in vitro fertilization divided in two groups implantation failure and success. After DNA amplification, samples were sent for sequence analyses, and rs5368 and rs3917458 were detected. The rs5368 and rs3917458 showed three genotypes. The three genotypes' distributions deviate from Hardy-Weinberg equilibrium. The rs5368 referred that T allele could be considered as a positive association with failure implantation, Odds ratio of rs3917458 show that the AA genotype and A allele was (2.21,2.26), so positive association with the failure implantation. Finally, SELE showed a low expression in women with failed implantation compared to women with successful implantation.

Keyword

SELE polymorphism, Gene expression, Implantation, IVF.

Embryo implantation into the endometrium is a highly regulated process that helps ensure a successful pregnancy [1]. Moreover, the window of implantation (WOI) is a specific time frame during which the maternal endometrium experiences drastic alterations known as "decidualization" and is optimal for blastocyst implantation [2]. The embryo, upon entering the uterine cavity, correctly orients itself toward the uterine epithelium, interacts with structures known as pinopodes, and initiates the processes of invasion and adhesion [3]. The quality of the embryo and the responsiveness of the endometrium are both crucial factors in successful implantation [4]. In fact, it has been hypothesized that a major cause of infertility is an endometrium that does not adopt a receptive phenotype [5]. In vitro fertilization (IVF) is a way to help women get pregnant when their fallopian tubes are damaged or blocked [6]. Therefore, improving technique need determine new factor

Could diagnostic and follow output of IVF as selectin. Adhesion molecules called selectins CD62 bind to the carbohydrate groups on adjacent cell surfaces via multiple transmembrane domains [7,8]. The selectin family is a group of mammalian vascular adhesion molecules that play a role in the adhesion

and deceleration of lymphatic and bloodstream cells on capillary endothelium [9]. Over the past few decades, selectins and their ligands have played a crucial role in human implantation [10,11]. Selectins (P-, L-, and E-selectins) are vital members of adhesion molecules regulating inflammatory and immune responses. They are mainly located in platelets, leukocytes, and endothelial cells [12]

E-selectin (SELE) belongs to the family of cell adhesion molecules known as selectins; it facilitates the recruitment, rolling, and migration of monocytes and lymphocytes to sites of inflammation [13]. In humans, the SELE gene on chromosome 1q12 codes for a 110-kDa carbohydrate-binding protein with 14 exons and 13 introns. [14]. This protein is typically expressed on activated platelets and endothelial cells. SNPs in the SELE gene, which codes for the E-selectin protein, have been associated with a variety of diseases. Many studies focused on the association of the SELE gene variation effect on the risk of various diseases such as breast cancer, atherosclerosis, metabolic syndrome in females, coronary artery disease, ischemic stroke, COVID-19 [15, 16, 17, 18, 19, 20]. In the present study, we study how variations

in the E-selectin gene and its expression level can affect implantation.

Methods

Design of Study

The ethics committee of Baghdad University's College of Science for Women approved the present research. At first, the aim of the study was explained to all participants, and after obtaining their oral and written consent, they were studied. The study included 100 women in an IVF cycle; all women were followed until a positive or negative pregnancy test. The sample divided into major groups: implantation failure female group and implantation success females group. Exclusion sample was (PGD, and male factor). Their age range was (18-44) years, from Rooh Alhayat Center for the treatment of infertility and IVF, AL Farah Specialist Fertility Center.

Collection of Samples:

Following embryo transfer, two milliliters of blood were drawn from each woman (success and failure groups). An EDTA tube was used to store the blood, and then all samples were stored in the freezer (-18°C) for molecular study.

Extraction of DNA and Genotyping:

The genomic DNA purification kit (Geneaid) was used to extract DNA from 200 liters of whole blood stored in EDTA tubes. In all samples, the genomic DNA was represented by bands in the gel electrophoresis indicating the presence of DNA. A total of 25 µL was used for the PCR amplification detection, which included 5 µL of genomic DNA, 13 µL of D.W., 5 µL of master mix, and 1 µL of each primer as follow forward primer were 5'-GCG CTACTT AGTTTTCA GCATGT-3'; and reverse were 5' ACTT GGTTACCT TGGGAAACG -3' were design by second author used primer designing tool. The program of PCR were shown in table 1. The 400 bp PCR fragment was confirmed to be present after separation on a 2% agarose gel with ethidium bromide staining. The Sequencing technique was used to identify the E selectin gene polymorphism.

Table (1) The PCR program of (SELE)

No	Step	Temperature °C	Time	No of cycle
1	Initial denaturation	94	5 min	1
2	Denaturation	94	30 sec	35
3	Annealing	58	30 sec	
4	Extension	72	1 min	
5	Final extension	72	5 min	1

DNA Sequencing

After amplification, the PCR products were analyzed of region for SELE gene (forward and reverse) of all failure and success implantation group. The 61 samples (35 failure group and 26 success group) were sent to MacroGen Corporation – Korea for sequencing by using automated DNA sequence MacroGen.

Real Time-PCR (RT-PCR)

RNA was isolated using the GENzolTMTriRNA Pure Kit, and then RNA was converted to complementary DNA (cDNA) using the AccuPower RRT RocketScript TPreMix Kit from Bioneer, Korea, and Oligo dT20 as primer. SELE gene expression was detected by real-time PCR technique (RT-PCR). This technique was carried out by a dye that used the real-time fluorescence of cDNA binding dye (SYBR Green) to measure cDNA amplification. E-selectin Primer for Real time PCR was forward CCTGCAATGTGGTTGAGTGT Gandreverse CTCGTTGTCCCAA TTCCAGA was designed by second author used primer designing tool. The required volume of each component was 25 µL: (5 µL SYPER Green), 13 µL nuclease-free water, 1 µL of each forward and reverse primer, and 5 µL cDNA. The absolute target quantities were calculated using the human reference gene (junctional cadherin complex regulator) (JHY). The primer of reference gene was forward GTCCAGG GGTATTA CAGGCAA and reverse TCAG GAA TCAGC CCAAGACG. The threshold cycle was used to quantitatively measure the levels of gene expression [21].

Statistical Analysis

We used IBM SPSS Statistics Version 22 to effect of various factors in study parameters. The results of genomic DNA amplification were analyzed using BioEdit software. Online Hardy-Weinberg equilibrium H.W.E Calculator to test whether the observed genotype are applied with H.W.E. WINPEPI software was used to calculate the significant and odds ratio of genotyping and alleles frequencies of studied genes.

Results

Results rs5368 C/T exon 9 Gene Polymorphism

The amplified DNA products 400 bp was shown in figure 1. Frequencies of genotypes and allele of the SNP of SELE T>C rs5368 using H.W.E was shown in table 2.

The results showed significantly higher ($P>0.05$) of genotypes frequency for both groups failure and success implantation. This indicates that the samples are deviations from H.W.E. law according to Chi square values ($X^2 = 24.77, 10.86$). The expected frequencies are as follows: the CC homozygous genotype recorded 28.35 vs. 23.09, while the heterozygous genotype CT has an expected frequency of 6.3 vs. 2.83, and the TT genotype has a 0.35 vs. 0.09 expected frequency among the two study groups. Homozygote genotype CC was more frequent in both groups, failure and success ($31 + 24 = 55$), which made it a common genotype in the Iraqi female population. Comparison of the frequencies of alleles and genotypes

SELE gene polymorphism (rs5368 T>C) between failure and success groups was shown in Table 3. The data show that the genotypes (CC, CT, and TT) were recorded differently between groups. Frequency homozygous genotype CC in success group (92.30%) than in failure group (88.57%), while the heterozygous

genotype CT was (3.85%) in success group and (8.57) in failure group. Homozygous TT genotype was higher in the success group (3.85) than in the failure group (2.86%).

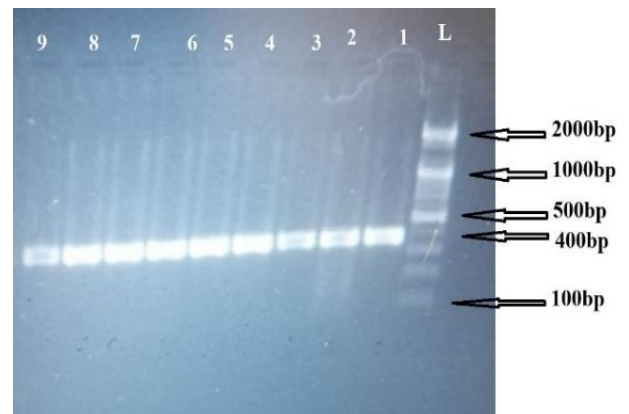


Fig. (1): SELE gene PCR product shown in gel. lane L, DNA Ladder marker. Lanes 1-10 study samples. DNA fragments 400 bps shown in gel

Table (2): Frequencies of genotypes and allele of the rs5368 using H. W.E

E selectin T>C rs5368		CC	CT	TT	C	T	χ^2	P-value
Failure implantation Genotype	Observed no (%)	31 88.57%	3 8.57%	1 2.86%	0.9	0.1	24.77 **	0.0001
	Expected no (%)	28.35 81%	6.3 18%	0.35 1%				
Success implantation Genotype	Observed no (%)	24 92.30%	1 3.85%	1 3.85%	0.94	0.06	10.86 **	0.004
	Expected no (%)	23.09 88.80%	2.83 10.88%	0.09 0.32%				
Total Observed no (%)		55 90.16%	2 3.28%	4 6.56%				
P value		0.345 NS	1.00 NS	0.317 NS				

Highly significant** ($P\leq 0.01$), NS: Non-Significant.

Table (3) Comparison of the Allele Frequencies and Genotype of SELE polymorphism (rs5368 T>C) between failure and success group.

SELE polymorphism rs5368 T>C	Frequencies (%)		Odds Ratio	Etiological or Preventive Fraction	Fisher's exact probability	CI 95%
	failure group (n=35)	success group (n=26)				
CC	88.57% (n=31)	92.30% (n=24)	0.65	35.4	0.8	(0.08 - 3.97)
CT	8.57% (n=3)	3.85% (n=1)	2.34	57.3	0.4	(0.23 - 64.02)
TT	2.86% (n=1)	3.85% (n=1)	0.74	26.5	0.7	(0.02 - 29.77)
Alleles Distribution						
C	49(92.86%)	65(94.23%)	0.8	20.4	0.8	(0.12 - 4.33)
T	3(7.14%)	5(5.77%)	1.26	20.4	0.8	(0.28 to 6.64)

The frequency of C allele was 49 (92.86%) in failure group compared with 65 (94.23%) in success group giving an odds ratio of 0.8 indicating that it may be considered as a protective allele while the odds ratio for the T allele was 1.26 suggesting that it might be considered as an etiological allele.

Results of Intron 8 (rs3917458 T>A)

Frequencies of genotypes and allele of the SNP of E Selectin T>A rs3917458 using Hardy- Weinberg

equilibrium was shown in table 4. The observed genotype frequencies were significantly higher than those predicted by H.W.E. (26.64 in the failure group and 20.94 in the success group; (Table 4); here is the data departure from H.W.E. The expected frequencies are: The AA homozygous genotype recorded 26.57 vs. 14.62, while the heterozygous genotype AT has an expected frequency of 7.84 vs. 9.75 and the TT genotype has a 0.58 vs. 1.62 expected frequency among the two study groups.

Table (4): Frequencies of genotypes and allele of thers3917458using (H.W.E).

E selectin Genotype T> A rs3917458		AA	AT	TT	A	T	χ ²	P-value
Failure implantation Genotype	Observed no. (%)	30(85.71%)	1 (2.86%)	4(11.43%)	0.87	0.13	26.64	0.0001
	Expected no. (%)	26.57(75.93%)	7.84(22.4%)	0.58(1.67%)				
Success implantation Genotype	Observed no. (%)	19(73.08%)	1(3.85%)	6(23.07%)	0.75	0.25	20.94 **	0.0001
	Expected no. (%)	14.62(56.25%)	9.75(37.5%)	1.62(6.25%)				
Total Observed		49(80.33%)	2(3.28%)	10(16.39%)				
P value		0.116 NS	1.00 NS	0.527 NS				

Highly significant** (P≤0.01), NS: Non-Significant.

The current study shown that frequency of homozygous genotype AA in success group was (73.08%) than in failure group (85.71%), while the heterozygous genotype AT and homozygous TT was(3.85%,23.07) in success group and (2.86,11.43) in failure group respectively(table5).The frequency of A allele was (87.14%)in failure group, while in success group was (75%).

Results of real time PCR of SELE

Sixty-one women undergone IVF (35 failure group implantation,26 success group) selected for E selectin gene expression. Q-PCR was validated and

performed. Selectin E gene expression was quantified into two groups: the failure group and the success group with the reference gene (JHY). As shown in Table 6 the mean ct of selectin E gene for the failure, and success group respectively (23.18, and 23.13), while the mean of ct for (JHY) gene expression for the failure, and success respectively (26.37 and 26.71). The present study result shows that the fold change in SELE was positive changed in failure group (0.763 ±0.26) than the success group (1.00 ±0.00) and no-significant difference in the fold gene expression between these two group, the failure and success group =0.602.P-value=0.294(Fig.2&b).

Table (5) Comparison of theAllele and Genotype Frequencies of E-selectin gene polymorphism (rs3917458 T>A) between failure and success groups

SELE polymorphism rs3917458 T>A	Frequencies (%)		Odds Ratio	Etiological or Preventive Fraction	Fisher's exact probability	CI 95%
	failure group (n=35)	success group (n=26)				
AA	30 (85.71%)	19 (73.08%)	2.21	54.8	0.2	(0.59 to 8.53)
AT	1 (2.86%)	1 (3.85%)	0.7	26.5	0.7	(0.02 to 29.77)
TT	4(11.43%)	6 (23.07%)	0.43	57.0	0.2	(0.10 to 1.79)
Alleles Distribution						
A	49(87.14%)	65(75%)	2.26	55.7	0.7	(0.87 to 5.96)
T	3(12.86%)	5(25%)	0.44	55.7	0.7	(0.17 to 1.15)

Table6: Comparison between Failure and Success folding of selectin E gene

Group	ct mean of SELE	ct mean of (JHY)	Δ ct mean	Δ ct mean of calibrator	Δ Δct	2-ΔΔct	Experimental group\success group	Fold of gene expression±Std
Failure group	23.18	26.37	-3.19	10.92	-14.11	17682.07	17682.07/23170.47	0.763 ±0.26
Success group	23.13	26.71	-3.58	10.92	-14.5	23170.47	23170.47/23170.47	1.00 ±0.00
t-test	--	--	--	--	--	--	--	0.602 NS
P-value	--	--	--	--	--	--	--	0.294

NS: Non-Significant.

Discussion

The current study detected the SELE gene at rs5368 and rs3917458. The failure and success groups

showed deviations from H-W-E in the genotypes of both SNPs. significant differences in (table 2,4) refer to effected of infertility in study population in which infertility in both group failure and success lead to deviate from HWE and these SNPs of SELE may be consider as a factor related with infertility. This

finding goes with study in Iraqi population that show that the observed genotype of rs5368 is deviate from HWE may be risk genotype for type 2 diabetes mellitus and breast cancer respectively [22,23,24]. The present study disagrees with Alzubadiy, show that SNP rs5367 C/T of SELE not related significantly with Diabetic Foot patients because all data still in agreement with H-W-E [25].

In the present study, polymorphisms of the E-selectin gene in rs5368 show that the odds ratio for the C allele was 0.8, so negative associations with failure of implantation according to the odds ratio could be considered a protective allele in Iraqi females. While the odds ratio for the T allele was 1.26 indicating positive association with the implantation which could be considered as an etiological allele. The current study agrees with a study in Chinese showing that the coal workers who had the SELE rs5368 CT genotype rather than the CC genotype were more likely to develop pneumoconiosis. [26]. Another study, on the other hand, found that SELE (rs5368) is associated with an increased risk of developing hypertension. C/C genotype of rs5368 and relative risks of hypertension in an East Asian population with C/T and T/T genotypes [27].

The SNPs rs3917458 show high odds ratio (2.21, 2.26) in AA genotype and A allele, which represent positive association with failure of implantation, and odds ratio for the AT and TT was (0.7, 0.43) respectively, which represent negative association with the implantation according to odds ratio could be considered as a protective genotype. Another study shows that the distributions of the A allele frequency of rs3917458 differed significantly between the hypertensive subjects and controls, so confer a risk of hypertension in the Han population [28].

The present study shows that SELE gene expression was decreased in the blood of failure groups (0.763) compared to success groups (1.00). These findings demonstrate that SELE lower expression may be associated with failure of embryo implantation. This result goes with a study that found that altered expression of selectins and their ligands was associated with infertility and abnormal pregnancies [29]. According to a recent local study, a gene called leukemia inhibitory factor receptor (LIFR) has been shown to have an effect on the success or failure of an IVF [30]. Another study shows that low expression of Integrin Beta3 is associated with uterine receptivity and plays a role in infertility [31,32].

Conclusion

In conclusion, SELE gene polymorphism associated with infertility. Allele T may be a risky allele of rs5368, while SNPs with rs3917458 may have allele A consider as risky related with failure of IVF. Strong

expression of SELE plays a strong role in the success of the implantation outcome. However, more studies are required to explain SELE gene expression in embryo implantation in uterus.

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In a declaration, the authors state that they have no financial or personal relationships that could be seen as compromising the independence of this study.

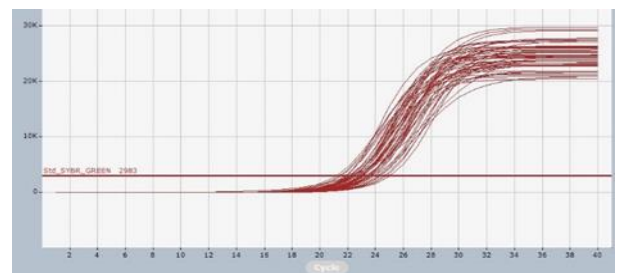


Fig. 2 a: SELE dissociation curves by qPCR. Samples included failure and success groups.

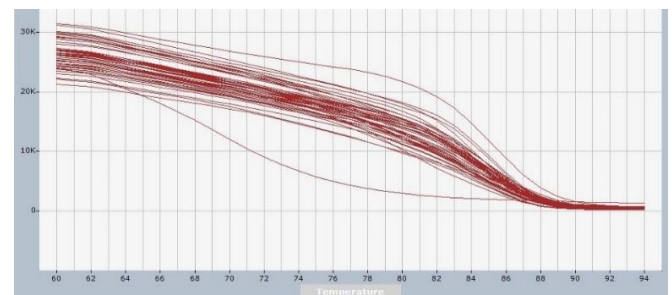


Fig. 2 b: SELE plots by qPCR. Samples of failure and success groups. This image directly from machine of qPCR.

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