Relation of Factor V Leiden Mutation to Repeated Implantation Failure in Women Undergoing Intra Cytoplasmic Sperm Injection (ICSI)

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Abstract

Objective: To determine whether women with factor V Leiden mutation, a common inherited defect of coagulation, are at increased risk for recurrent pregnancy loss after ICSI.

Patients and Methods: Women with recurrent implantation failure were compared to a matching group of women conceived naturally. Factor V Leiden was investigated in each group to determine its homozygosity or heterozygosity and its relation and effect on implantation and ICSI. Study of Factor V was done through its purification, then amplification and detection using real-time PCR (Polymerase Chain Reaction).

Results: No statistical significant difference was found between FVL mutation in cases of repeated implantation failure and controls who achieved natural conception (OR: 0.190 in cases and 0.333 in controls). Also No association were found in Age, BMI, Prevelance of Hypertension, History of DVT between cases with repeated implantation failure and controls who achieved natural pregnancy.

Conclusion: It seems there is no association between Factor V Leiden mutation gene and repeated implantation failure in women undergoing ICSI, and so routine anticoagulant use for these women is not recommended.

Key Words: Factor V leiden – Thrombophilia – ICSI.

Introduction

THROMBOPHILIA (including FV Leiden mutation) has been attributed to many cases of recurrent pregnancy loss [1-7]. Also many studies investigated the possible factors affecting recurrent implantation failure or IVF-ET failure, these include: Age of the mother, her parity, her basal hormonal level, inherited thrombophilia or history suggestive or favoring a thrombophilic disorder, also factors related to the uterus as: Endometrial thickness, position and length of the uterus and last, but not least, the technique of Embryo Transfer [8-13]. The inherited thrombophilia is known to affect the initial vascularization at the implantation site, and though the success of normal pregnancy and embryo development [14-16]. This process is considered as a rate-limiting step for the establishment of a successful implantation and pregnancy following ICSI. [17] The present study was conducted to determine the relation of Factor V Leiden mutation and its effect on repeated implantation failure, by comparing its level in cases of repeated implantation failure following ICSI and other cases with normal pregnancy.

Patients and Methods

Cases included twenty five women referred to infertility center in Cairo to undergo ICSI, these women were between 18 and 35yrs old. Women having hydrosalpinx, uterine anomalies, uterine fibroids, current use of anticoagulant medications, were excluded from the study. All women underwent ICSI with: Successful ovarian stimulation (all using the long protocol), oocyte retrieval, fertilization and transfer of 2 fresh embryos grade 2, prepared from fresh sperms. Women were followed until the time of uterine ultrasound examination to determine whether or not implantation had occurred.

Twelve control women were also included in this study. These women conceived naturally (without ovarian stimulation) and gave birth to healthy newborns. All cases and controls had same ethnic origin, they all underwent full history taking (medical, obstetric, thrombotic and surgical), through general examination, full routine laboratory work-up (CBC, LFTs, KFTs, Coagulation profile, Lupus Anticoagulant, Anticardiolipin IgG and IgM., and Factor V Leiden testing.

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All cases and controls were medically free (no diabetes, hypertension, cardiac or renal troubles). Only one of the cases gave history of DVT 3yrs back. Three of the cases gave history of previous abortions, while one of the controls gave history of previous abortion. The study was approved by the Ethical Committee of Kasr Al Aini at Faculty of Medicine, Cairo University, Egypt.

The Laboratory Assay procedure of Factor V Leiden Kit was done in Genomic Center of Kasr Al Aini between December 2010-December 2011:

- Purification: Genomic DNA is extracted from whole blood samples using the Manual specimen preparation procedures of High Pure PCR Template Preparation Kit which purchased from Roche Diagnostic manufactured in Germany.

- Amplification and detection by real-time PCR: The Factor V Leiden is an in vitro diagnostic test for the detection and genotyping of the Factor V Leiden mutation as an aid to diagnosis in the evaluation of patients with suspected thrombophilia [18,19]. A point mutation at position 1691 (G to A at position 1691) of the factor V gene causes an arginine to glutamine substitution at position 506 in the Factor V protein. The test is performed on the LightCycler 2.0 Instrument (Roche Diagnostic-Germany) utilizing polymerase chain reaction (PCR) for the amplification of fragment of the Factor V gene from human genomic DNA using specific primers. The amplicon is detected by fluorescence using a specific pair of probes, which consist of two different oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle.

The hybridization probes were used to determine the genotype by performing a melting curve analysis after the amplification cycles were completed. The red labeled hybridization probe, acts as an anchor probe that hybridizes to a part of the target sequence that was not mutated, while the fluorescein labeled probe (mutation probe) spans the mutation site. During the melting curve analysis, increasing temperature causes the fluorescence to decrease since the shorter of the 2 probes (mutation probe) dissociates first, and the 2 fluorescent dyes are no longer in close proximity. If FV Leiden (G1691A) mutation is present, the mismatch of the mutation probe with the target destabilizes the hybrid so the decrease in fluorescence, will occur at a lower temperature (57 ± 2.5 °C) while with the wild type genotype, mismatch will not occur, and therefore, the DNA has a higher melting temperature (65 ± 2.5 °C). The heterozygous genotype exhibits a distinctive combination of properties as shown in Figs. (1,2).

Data were statistically described in terms of Mean ± Standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Odds Ratio (OR) and 95% Confidence Interval (95%CI) were calculated for FLV mutation between cases and controls. Comparison of numerical variables between the study groups was done using Student t-test for independent samples. For comparing categorical data, Chi square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. p-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.
Results

Twenty-five cases with repeated implantation failure were analysed regarding their FV Leiden, to determine its homo or heterozygosity, this group is compared to a matching group of twelve patients who had normal conception. Both groups had similar demographic characteristics regarding the age, BMI, all were of the Caucasian race, seeking the same hospital (so of same socioeconomic standard).

There was no statistical difference between both groups regarding the age (p-value: 0.103) also, by comparing the wild and heterozygous cases regarding FVL, there was no statistical difference (p-value: 0.090).

As regard BMI, there was no statistical significant difference between BMI of both groups (p-value: 0.745). Also there was no statistical significant difference between the wild types and the heterozygous among the cases (p-value: 0.893). These data (regarding the age and BMI differences) are shown in (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Cases (nb: 25)</th>
<th>Controls (nb: 12)</th>
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<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>Heterozygous</td>
</tr>
<tr>
<td></td>
<td>(nb:21)</td>
<td>(nb:4)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>34±5.244</td>
<td>29±4.690</td>
</tr>
<tr>
<td>p-value</td>
<td>0.090</td>
<td>0.103</td>
</tr>
<tr>
<td>BMI</td>
<td>28±5.020</td>
<td>28.35±1.7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.893</td>
<td>0.745</td>
</tr>
</tbody>
</table>

Table (1): Difference in Age and BMI between the 2 groups.

There were three out of the 25 cases suffering from hypertension (14.3%), while in the control group, none of them had hypertension. Among these 3 cases, all of them were of the wild type (none homo or heterozygous regarding FVL). By analyzing these data, the p-value was 0.537, showing that there was no statistical significant difference between the cases and the controls regarding the hypertensive status, so hypertension doesn’t play a role in determining the repeated implantation failure according to the current study.

Also three out of the 25 cases had history of DVT (14.3%), these 3 cases were of the wild type, and none of the controls had history of DVT, showing that DVT doesn’t play a role in affecting the implantation in cases of ICSI according to the current study (p-value: 0.537).

Table (2) analyses the number of both cases and controls, and whether the patients were homozygous, heterozygous or of normal zygosity. In our results, we didn’t have any of the homozygous type (whether in cases or control groups). But we have 4 out of 25 of the heterozygous type (16%). While among the controls, we had 3 out of twelve of the heterozygous type (25%). These results showed that there were no statistical significant difference between the genetic analysis of FVL (whether homozygous, heterozygous or wild types) between the 2 groups (as determined by the p-value: 0.659) and so FVL.

So FVL gene showed a mutation in 4 out of 25 in cases, and 3 out of 12 in controls yielding to an odd of 0.190 in cases and 0.333 in controls with and OR (odds ratio of 0.571) with a 95% CI of 0.106-3.092. according to this study, doesn’t play a role in recurrent implantation failure in patients undergoing ICSI, as its genetic analysis is the same in group having normal conception and the group having repeated implantation failure after ICSI.

Table (2): Analysis of FVL gene by PCR.

<table>
<thead>
<tr>
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<th>Cases (nb: 25)</th>
<th>Control (nb: 12)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Heterozygous</td>
<td>Wild</td>
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<tr>
<td></td>
<td>type</td>
<td>type</td>
</tr>
<tr>
<td>Nb</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>Percentage</td>
<td>16%</td>
<td>84%</td>
</tr>
<tr>
<td>p-value</td>
<td>0.659</td>
<td>0.190</td>
</tr>
<tr>
<td>Odds</td>
<td>571</td>
<td>(0.106-3.092)</td>
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<td>OR (95% CI)</td>
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Discussion

Failure of implantation of the fertilized embryo in the endometrium after ICSI procedure remain major concern exam after all the technological advancement in ICSI technology in recent years [20] One of the postulated mechanism responsible for implant failure after ICSI is hypercoagulability due to the presence of thrombophilia which included point mutation in the gene encoding coagulation factor V due to substitution of guanine by adenine at nucleotide 1691 [21].

It was hypothesized that hypercoagulability in the mother, leading to an impairment of the uteroplacental circulation leading to unsuccessful embryo implantation [21] In the present study, 37 women were included (25 cases with FVL mutation gene defect, and 12 controls with normal conception), in both groups, we concentrated on the effect of age, BMI, Hypertension, history of DVT and lastly and most important the Factor V Leiden
mutation, and their effect on repeated implantation failure or the occurrence of natural conception.

Women below the age of 41 have improved implantation, but a high cancellation rate and low cycle outcome than in older women [22,23], in our study the cases had a mean age of 33yrs, while the controls had a mean age of 30yrs, showing that pregnancy is better in younger age, but the difference wasn’t statistically significant (p-value: 0.103)

In the present study, the mean BMI (Body Mass Index) for cases was 28.50, while the mean BMI of the controls was 28.58. The difference in BMI isn’t statistically significant (p-value: 0.745). None of the controls had hypertension, while 3 out of 25 cases (88%) suffered from hypertension. These results didn’t show any statistical significance as the p-value is 0.537. None of the controls suffered from DVT, while 3 of the cases had history of DVT (88%), with a p-value of 0.537, showing no statistical significant value.

In the present study, 3 out of the 12 controls showed FVL mutation (they were heterozygous), while 4 out of the 25 cases showed FVL mutation (they were heterozygous also). Our results show no statistical significant difference between FVL gene in cases with repeated implantation failure and cases with normal implantation and conception. (p-value: 0.659, showing also no statistical significant difference between both groups.

Our results are comparable with Martinelli et al., [24] who showed that failure of pregnancy following IVF-ICSI isn’t associated with increased possibility with thrombophilia in the patients. But our results are different from Grandone et al., who investigated also a small number of patients (forty two), as they found an association between FVL mutation and repeated implantation failure and so they indicated the use of anticoagulant prophylaxis at the time of Assisted Reproductive procedure to improve the implantation rate [28]. Also our results are different from Azem et al., who suggest that inherited thrombophilia may play a role in the etiology of repeated IVF failures, particularly in the subgroup with unexplained fertility, this after investigating 45 women with repeated implantation failure and unexplained infertility. They showed that the incidence of thrombophilia in women with unexplained infertility in group A was 42.9% (9/21), compared with 18.2% in group B (p<0.002) [26].

There are some limitations in our study, first, the small sample size of the studied population, this is due to the expensive kits of FVL in our limited resource country. Second, the odds ratio of repeated implantation failure is statistically not significant (OR: 0.571), this may suggest the weak association between FVL Mutation and repeated implantation failure, therefore a larger sample size study is recommended in order to obtain a statistical significance. Third, it could been argued that a control group of women who conceived after IVF-ICSI would be more appropriate, however as few of these women have successful embryo implantation (4 out of the cases), so it wasn’t practical to obtain the cases and the controls from the same group.

From this study we concluded that, Thrombophilia in the form of FVL mutation doesn’t lead to repeated implantation failure. Still the role of FVL should be assessed in a larger sample size. But according to this study, and lack of relation between thrombophilia and repeated implantation failure doesn’t support the routine prophylaxis use of anticoagulant treatment in women undergoing IVF-ICSI.

References


