Effect of Polymorphisms of Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor Beta-1 (TGF-β1) in Women with Recurrent Miscarriage

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Abstract

The current study was conducted in the laboratories of Thi-Qar University's Research and marshes center in collaboration with (Bint Al Huda Hospital) from February 2023 to August 2023. The aim of the current study was to investigating the allelic association of a single nucleotide polymorphism in VEGF and TGFβ1 genes with Recurrent miscarriage incidence by using PCR and gel electrophoresis. The study included 100 human blood samples with recurrent miscarriage women and 50 human blood samples as control group their ages ranged were between 17- 45 years. The patients and control were divided into three groups according to their age stages. The first group aged 17-26 years, the second group aged 27 – 36 years, and the third group aged 37- 46 years. The VEGF and TGFβ1 promoter genes polymorphism (182 G/C) (488 C/T) promoter gene polymorphism was identified, and genotyping was verified by direct sequencing. The results of the statistical analysis showed no correlation between the occurrence of recurrent miscarriage and the emergence of mutation VEGF and TGFβ1 genes at p. value < 0.05. when it was compared with the control in some population of Thi-Qar province. Conclusions This research confirms that polymorphisms in the VEGF and TGFβ1 genes have no impact on the prevalence of RM in Thi-Qar communities.

Key words: Recurrent Miscarriage, VEGF gene, TGF-β1 gene, polymorphism-PCR

1. Introduction

Miscarriage is defined as an incomplete pregnancy and the loss of the fetus in the first few months of pregnancy [1]. It can be classified as a threatening miscarriage, an inevitable miscarriage, a full miscarriage, a missed miscarriage, an incomplete miscarriage, an abortion paired with an infection and, a recurring miscarriage based on the clinical symptoms and various phases of development.
When two or more pregnancies end before 20 weeks of gestation, it is referred to as a recurrent miscarriage (RM) or recurrent pregnancy loss (RPL) [2]. There are two categories of miscarriages: primary and secondary. According to Nielsen (2011) [3], a main miscarriage occurs when there are many consecutive abortions, but a secondary miscarriage occurs after a successful pregnancy and continues from there. Fetal tissue analysis can determine if an early pregnancy loss is caused by an aberrant embryo or a genetically altered fetus [4].

Genetics, structural anomalies, infections, immunological variables, and endocrine disorders are associated with recurrent abortion [5]. Thrombophilia's, aberrations, chromosomal abnormalities, endocrinological disorders, sperm DNA fragmentation, immunological and immunogenetic causes, epigenetic causes, failure of embryo selection, uterine malformations, and lifestyle factors are among the causes of recurrent miscarriages, according to (Sultana and Nallari, 2020) [6]. Abortion causes are numerous and include viruses, protozoa infections, cervical incompetent pregnant women, ischemia and hypoxia, aberrant blood sugar increase, abnormal thyroid function, and poor lifestyle choices such drug and alcohol abuse, smoking, and drinking [7].

Growth factors are signaling proteins that promote cell division, growth, interaction, and control. Growth factors are essential for the regulation of inflammation, control of proliferation, differentiation of various cell types, angiogenic processes, neuronal function, tissue regeneration, and embryonic development. Vascular endothelial growth factor (VEGF), is a crucial component in the development of capillaries. Nearly all organs generate VEGFA, which promotes endothelial cell proliferation and vascular [8]. VEGF is necessary for several processes in the early stages of pregnancy, including trophoblast proliferation, oocyte maturation, embryo implantation and development, placenta angiogenesis, and the establishment of maternal and fetal blood arteries in the uterus.

The pathophysiology of RM in women may include VEGF. Functional VEGF gene polymorphisms have been linked to an increased risk of developing RM, according to several prior investigations [9].

Transforming growth factor βeta-1 (TGF-β1) suppresses the migration and proliferation of endothelial and smooth muscle cells while promoting the healing of wounds. The body generates TGFβ and its receptor in almost all cells. On the other hand, TGF-β1 s inhibits the production of pro-inflammatory cytokines, the immune system, hematopoiesis, and the development of deadly natural killer cells (NK) and T lymphocytes. It has been demonstrated that blastocyst cells produce TGF-β1.

NK cells and decidual stem cells subsequently generate TGFβ, which is required for trophoblast formation and expansion. During the third week of pregnancy, the first indications of placental angiogenesis are seen. Chorionic gonadotropin is highly expressed by cytotrophoblast cells, and this has a beneficial impact on angiogenesis and endothelial vascular growth factor expression. Remodeling of the spiral arteries is essential for healthy fetal growth and development. Pregnancy loss is typically the result of violations of the present implantation phases and fetal maternal blood formation [8].
2.1. Sample Collection

This study was conducted in the laboratories of Thi-Qar University's Research and marshes center in collaboration with Bunt Al-Huda Hospital Teaching Hospital, in the period from February 2023 to August 2023. The study included (150) samples, (100) of them were recurrent miscarriage patients divided into three groups according to age as in the following: First group from 17 - 26 years and their number 38 miscarried women, Second group from 27 - 36 years and their number 42 miscarried women and third group from 37 - 45 years and their number 20 miscarried women and (50) subjects as a control. Blood samples were obtained from all samples. Five milliliters were put directly in a sterile tube containing EDTA for DNA extraction.

2.2. DNA Extraction

Genomic DNA (gDNA) was isolated from Peripheral Blood Leukocytes by using a kit provided by Geneaid/Taiwan. A spectrophotometer (Nanodrop, Thermo Scientific, USA) was used to measure the amount and quality of DNA (ng/microliter) by reading the absorbance (260/280 nm) in accordance with the manufacturer's instructions. Polymerase Chain Reaction (PCR), amplification of DNA for VEGF and TGFβ1 genes. The following cycling conditions were used: 95°C for 5 min; 38 cycles of (95°C, 30 sec), (60°C, 30 sec), (72°C, 45 min); followed by 72°C for 7 min.

Table 1: List of Primers used for PCR amplification.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF[10]</td>
<td>Forward</td>
<td>5'TTGCTTGGCCATTTCCCACCTGA -3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-CCGAAGCGAGAACAGCCCAGAA-3’</td>
</tr>
<tr>
<td>TGFβ1[11]</td>
<td>Forward</td>
<td>5’- GCAGTTGGCGAGAACAGAAGTGG-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’- CCAGAAGCAGAAGAGTCAG-3’</td>
</tr>
</tbody>
</table>

Table 2: PCR reactions with respective annealing temperatures utilized for DNA amplification.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Product size (bp)</th>
<th>Tm (degree)</th>
<th>Cycle conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>469 bp</td>
<td>60°C</td>
<td>38</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>597 bp</td>
<td>62°C</td>
<td>38</td>
</tr>
</tbody>
</table>

All samples should have a proliferative fragment of 469 bp and 597 as a PCR accuracy check. The normal genotype (GG, CC), as well as the mutant genotypes (GC, CT), were included in the polymerase chain reaction (PCR) products that were anticipated for this investigation. Following PCR, the products were electrophoretically separated on an agarose gel, and bands were then seen using ultraviolet vision (Figure.1&2).

3. Statistical Analysis

The results of the current study were analyzed using Chi-square test, and Odds ratio to detect the frequency of genotypes for the VEGF, TGFβ1 genes using the SPSS statistical analysis program.
4. Results and Discussion

4.1. Demographical Study:

4.1.1. Age Distribution in Miscarriage Women and Control Group

The current results recorded the high miscarriaged women in the second age group 42%, while the lowest miscarriaged women in the third age group 20%. In contrast, the high women in the control group in the first age group 50%, and the lowest in the first age group 16%, the results also, showed a non-significant difference between miscarriaged women and control group in age groups and in mean of the age at p. value < 0.05.

Table 3: Show distribution of study groups according to Age

<table>
<thead>
<tr>
<th>Age Groups in years</th>
<th>Patients</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>17 - 26</td>
<td>38</td>
<td>38.0</td>
<td>25</td>
</tr>
<tr>
<td>27 - 36</td>
<td>42</td>
<td>42.0</td>
<td>17</td>
</tr>
<tr>
<td>37 - 45</td>
<td>20</td>
<td>20.0</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>66.67</td>
<td>50</td>
</tr>
</tbody>
</table>

Cal$X^2$ = 2.923 Tab$X^2$ = 5.99 DF= 2 p. value 0.232

Age means

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29.2 ± 7.35</td>
<td>27.9 ± 6.94</td>
</tr>
<tr>
<td>p. value</td>
<td>0.323</td>
<td>0.323</td>
</tr>
</tbody>
</table>

Age-specific differences in the miscarriage rate have been observed based on the demographic distribution of patients and the control group. They were greatest in the patients' age group of 27 to 36 years, next in the age group of 17 to 26 years, and lastly in the age group of 37 to 45 years. Up till the current study's results demonstrated that the age group has no bearing on miscarriage in regards to the distribution of miscarriages by age group. The current study's findings were in odds with $^{[12]}$. It was shown that a woman's fertility decreases as she ages, and woman with ages of between 35 and 40. This may be associated to conditions including endometriosis, anovulation, and pelvic infections. factors other than aging may contribute to reproductive issues. Fetal chromosomal abnormalities in older pregnant women are the most prevalent cause of recurrent miscarriages, and they are linked to women's progressively delaying childbearing until their late 30s and early 40s in many nations. Compared to younger moms, older women produce fewer high-quality eggs, which increases the likelihood of chromosomal abnormalities and miscarriage $^{[13]}$.

4.1.2. Distribution of Miscarriaged Women and Control Group According to Level of Education

The current results recorded the miscarriaged women decreased with increasing of level of education were high in literacy women 40% and the lowest in in women with university education 10%. In contrast, the high women in the control group in primary education 52%, and the lowest in women with university education 6.0%, the results also, showed a significant difference between miscarriaged women and control group according to level of education at p. value < 0.05.
Table 4: Distribution of miscarriaged women and control group according to Level of Education

<table>
<thead>
<tr>
<th>Level Education</th>
<th>Patients</th>
<th></th>
<th>Control</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Literacy</td>
<td>40</td>
<td>40.0</td>
<td>7</td>
<td>14.0</td>
<td>47</td>
<td>31.33</td>
</tr>
<tr>
<td>Primary</td>
<td>32</td>
<td>32.0</td>
<td>26</td>
<td>52.0</td>
<td>58</td>
<td>38.67</td>
</tr>
<tr>
<td>Middle</td>
<td>18</td>
<td>18.0</td>
<td>14</td>
<td>28.0</td>
<td>32</td>
<td>21.33</td>
</tr>
<tr>
<td>University</td>
<td>10</td>
<td>10.0</td>
<td>3</td>
<td>6.0</td>
<td>13</td>
<td>8.67</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>66.67</td>
<td>50</td>
<td>33.33</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

CalX^2 = 20.454    TabX^2 = 7.81    DF = 3    p. value < 0.01**

Additionally, the study's findings demonstrate that there were 60 miscarried women patients from educated women, compared to 40 miscarried woman patients from uneducated women. We discovered that the majority of educators live in cities, which may have an impact on the prevalence of inoperative among urban inhabitants and the environmental circumstances in cities, explaining the high percentage of educated women. This finding is consistent with [14], who has indicated that no association was seen between miscarried women and a low educational level.

4.1.3. Distribution of Miscarriaged women and Control Group According to Family History

The current results noted the high miscarriaged women in those non family history 64%, while the lowest miscarriaged women in those with family history 36%. Also, the high women in the control group in those non family history 90%, and the lowest in those with family history 10%, the results also, showed a significant difference between miscarriaged women and control group according to family history at p. value < 0.05.

Table 5: Distribution of miscarriaged women and control group according to family history

<table>
<thead>
<tr>
<th>Family History</th>
<th>Family History</th>
<th>Non-family History</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Patients</td>
<td>36</td>
<td>36.0</td>
<td>64</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>27.33</td>
<td>109</td>
</tr>
</tbody>
</table>

CalX^2 = 19.058    TabX^2 = 3.84    DF = 1    p. value < 0.01**

According to the present study's findings, 36% of miscarried status holders had a familial history. However, 64% of miscarried statuses do not have a familial history. Consequently, a smaller proportion
of patients with miscarried women have a family history than do those without. This conclusion is consistent with [15], which states that miscarried women are more likely to report family history, even if it is from a more distant cousin. This finding is likely due to the fact that miscarried women are a sickness with a variety of reasons. Moreover, first-generation relatives have a higher biological plausibility for a family relationship. This indicates that even though a substantial correlation was discovered, further study is required to pinpoint the precise pathways through which family risk is passed down, such as through mothers, grandparents, or other female relatives, as well as through paternal influence. It is estimated that more than 5% of couples who experience repeated miscarriages also have chromosomal abnormalities in themselves, which may raise the likelihood that the fetus may have abnormalities that lead to miscarriage or recurrent miscarriages [16]. This research, however, disagrees with [17], which states that while a family history of miscarriage is not yet a well-recognized predictor of miscarriage, there is mounting evidence to support a possible familial relationship.

4.1.4. Distribution of Miscarriaged Women and Control Group According to Presence of other Disease

The current results noted the high miscarriaged women in those infected with other disease 58%, while the lowest miscarriaged women in those non other disease 42%. In other hand, the high women in the control group in those non other disease 86%, and the lowest in those non disease 14%, the results also, showed a significant difference between miscarriaged women and control group according to other disease at p. value < 0.05.

Table 6: Distribution of miscarriaged women and control group according to presence of other disease

<table>
<thead>
<tr>
<th>Other diseases</th>
<th>Other diseases</th>
<th>Non-other diseases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Patients</td>
<td>58</td>
<td>58.0</td>
<td>42</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>14.0</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>43.33</td>
<td>85</td>
</tr>
</tbody>
</table>

The present study's findings demonstrated a strong association between patients who experienced miscarriages and both the control group and the patients. Existence of additional illnesses is consistent with this result [18]. In women with polycystic ovarian disorder, at least 40% of pregnancies generated by clomiphene end in spontaneous abortions. Clomiphene's antiestrogen activity may be the source of this high incidence. Its lengthy half-life, may result in improper progesterone synthesis during the luteal phase. Additionally, it has been noted that infections in embryos and fetuses cause (RM) at an incidence of less than 4%.
4.2. Molecular Study

- Figure (1): Product size for VEGF Gene (469 bp)
- Electrophoresis Protocol (TBE, Agarose Gel 1.5%, 70 volt & 85 mA for 40 min)
- Orange Dimond Dye \textsuperscript{int} “Promega” – Ladder 100 bp “Bioneer”
- Electrophoresis Device & Power supply "Cleaver UK"

4.2.1. Frequency of genotypes for the VEGF gene samples of patients and controls.

The study recorded a non-significant difference in the frequencies of the C/C, G/C, and G/G genotypes in aborted women compared with the control group, and non-significant difference in the frequencies of the C and G alleles. While the odds ratio for frequency the allele in aborted women compared to the control group was recorded at p. Value < 0.05 as in Table 7

Table 7: Distribution of genotypes for the VEGF gene samples of patients and control.

<table>
<thead>
<tr>
<th>VEGF Gene</th>
<th>Genotype</th>
<th>Patients No. 100</th>
<th>Control No. 50</th>
<th>CalX(^2) p. value</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G182C</td>
<td>C/C</td>
<td>12 12.0</td>
<td>9 18.0</td>
<td>0.215</td>
<td>Non-OR</td>
</tr>
<tr>
<td></td>
<td>G/C</td>
<td>45 45.0</td>
<td>25 50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>43 43.0</td>
<td>16 32.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patients</th>
<th>Control</th>
<th>CalX(^2)</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>57 39.31</td>
<td>34 35.79</td>
<td>0.661</td>
<td>1.13 (0.64 - 2.0)</td>
</tr>
<tr>
<td>G</td>
<td>88 60.69</td>
<td>41 64.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The current findings demonstrated that the mutant heterozygous C/G and the mutant homozygous C/C do not differ significantly from one another. These findings suggest that there is no association between VEGF gene variation and miscarried women. These findings refute the pathophysiological role of the VEGF gene in the miscarriage in Thi-Qar community patients. The research concurs with \cite{19}, and with others who said that no correlation has been observed across several populations. However, these results differ from those of \cite{20}, initially used the r polymorphism locations of the VEGF gene to report a
connection with RM. A stronger correlation with RSA was discovered in the Greek population. Increased RM was linked to VEGF GC polymorphism mutation in both Asian and Caucasian groups.

4.2.2. Association between VEGF Genotypes and Age of Miscarriaged Women

The current study reported the high genotypes was GC in second age group, while the lowest genotype was CC in the second age group, the results also noted a non-significant difference between classes of genotypes and age of patients, at p. value < 0.05.

Table 8: Association between VEGF genotypes and age of miscarriaged

<table>
<thead>
<tr>
<th>VEGF genotype</th>
<th>CC</th>
<th>GC</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>17 – 26</td>
<td>2</td>
<td>2.0</td>
<td>17</td>
</tr>
<tr>
<td>27 – 36</td>
<td>4</td>
<td>4.0</td>
<td>21</td>
</tr>
<tr>
<td>37 – 45</td>
<td>6</td>
<td>6.0</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>12.0</td>
<td>45</td>
</tr>
</tbody>
</table>

CalX² = 8.52 TabX² = 9.49 DF= 4 p. value 0.074

According to the results, the highest genotype for the second age group is GC, while the lowest genotype is CC. The results also showed that there were no significant differences in the genotype categories according to the ages of the patients. These results did not support previous data that indicated a positive linear relationship between VEGF levels and maternal age within the RPL group [21].

4.2.3. VEGF Genotypes and Family History in Miscarriaged Women

The current study showed that the highest genotype was GG in women with no family history, followed by the CC genotype and then the GC genotype. The results also indicated that there was a non-significant difference between the categories of genotypes and family history, at p. Value <0.05.

Table 9: Association between VEGF genotypes and family history in miscarriaged women

<table>
<thead>
<tr>
<th>Family History</th>
<th>VEGF genotype</th>
<th>Family History</th>
<th>Non-family history</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>CC</td>
<td>5</td>
<td>41.67</td>
<td>7</td>
<td>58.33</td>
</tr>
<tr>
<td>GC</td>
<td>19</td>
<td>42.22</td>
<td>26</td>
<td>57.78</td>
</tr>
<tr>
<td>GG</td>
<td>12</td>
<td>27.91</td>
<td>31</td>
<td>72.09</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>36.0</td>
<td>64</td>
<td>64.0</td>
</tr>
</tbody>
</table>

CalX² = 5.585 TabX² = 5.99 DF= 2 p. value 0.061
Previous study indicated there was no statistically significant difference in the genotype class and family history between patients with and without a family history [22]. Serum VEGF levels were shown to be lower in individuals with a history of RPLs than in those with normal pregnancies. On the other hand, women who have a history of repeated spontaneous abortions account for 60% of abortions; this may be because of chromosomal defects, numerous environmental variables, or hereditary diseases in miscarried individuals [23]. Additionally, it was shown that there are no genotypes or allele frequencies for the VEGF SNPs studied in the chorionic tissue in first-trimester miscarriages [19].

### 4.2.4. Frequency of genotypes for the TGF-β1 gene of patients and controls.

The current study recorded a significant difference in frequencies of C/C, C/T and T/T genotypes in miscarried compared with control group. While, noted a non-significant difference in frequencies of C and T allele. Also, recorded by Odds ratio a non-significant difference in frequency of allele in miscarried women than control group at p. value < 0.05.

**Table 10: Distribution of genotypes for the TGF-β1 gene samples of patients and healthy controls.**

<table>
<thead>
<tr>
<th>TGF-β1 Gene</th>
<th>Genotype</th>
<th>Patients No. 100</th>
<th>Control No. 50</th>
<th>CalX² p. value</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.    %</td>
<td>No.    %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C488T</td>
<td>C/C</td>
<td>30   30.0</td>
<td>16   32.0</td>
<td>0.023⁺</td>
<td>Non-OR</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>43   43.0</td>
<td>28   56.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>27   27.0</td>
<td>6    12.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Figure (2): Product size for TGFBI Gene (597 bp)
- Electrophoresis Protocol (TBE, Agarose Gel 1.5%, 70 volt & 85 mA for 40 min)
- Orange Dimond Dye mt "Promega" – Ladder 100 bp "Bioneer"
- Electrophoresis Device & Power supply "Cleaver UK"
Research has shown that the heterozygous C/T mutant and the homozygous C/C mutant differ significantly from each other, as these results indicate a relationship between miscarried women and TGF-β1 polymorphisms. [24] TGF β1 polymorphism raises Tunisian women's likelihood of experiencing repeated miscarriages. This polymorphism was not linked to miscarriage in the Caucasian population [25], nor was it linked to miscarriage in the Brazilian population [8].

4.2.5. Association between TGF-β1 Genotypes and Age of Miscarriaged Women

The current study reported the high genotypes was CT in first age group, while the lowest genotype was CC and TT in the third age group, the results also noted a non-significant difference between classis of genotypes and age of patients, at p. value < 0.05

Table 11: Association between TGF-β1 genotypes and age of miscarried

<table>
<thead>
<tr>
<th>TGF-β1 genotype</th>
<th>CC</th>
<th>No.</th>
<th>%</th>
<th>CT</th>
<th>No.</th>
<th>%</th>
<th>TT</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 – 26</td>
<td>12</td>
<td>12.0</td>
<td>19</td>
<td>19.0</td>
<td>7</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 – 36</td>
<td>12</td>
<td>12.0</td>
<td>16</td>
<td>16.0</td>
<td>14</td>
<td>14.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 – 45</td>
<td>6</td>
<td>6.0</td>
<td>8</td>
<td>8.0</td>
<td>6</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30.0</td>
<td>43</td>
<td>43.0</td>
<td>27</td>
<td>27.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CalX² = 2.49 TabX² = 9.49 DF= 4 p. value 0.645

The current study found that the first age group had the highest CT genotype, while the third age group had the lowest CC and TT genotype. The results also showed that there was no significant difference between genotype category and patient age; This is likely due to the small sample size and inaccurate information on patient age. On the other hand, the rate of recurrent miscarriage increases with the mother's age due to chromosomal abnormalities in the children or genetic defects in the sperm or egg that cause miscarriage. Furthermore, recurrent miscarriages are more common when the father is older, which is a risk factor.

4.2.6. Association between TGF-β1 Genotypes and Family History in Miscarriaged Women

The current study showed that the highest genotype was CC in women with no family history, followed by the CT genotype and then the TT genotype. The results also indicated that there was a non-significant difference between the categories of genotypes and family history, at p. Value <0.05
Table 12: TGF-β1 genotypes and family history in miscarried women

<table>
<thead>
<tr>
<th>Family History</th>
<th>GTF β1 genotype</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>9</td>
<td>30.0</td>
<td>21</td>
<td>70.0</td>
<td>30</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>16</td>
<td>37.21</td>
<td>27</td>
<td>62.79</td>
<td>43</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>11</td>
<td>40.74</td>
<td>16</td>
<td>59.26</td>
<td>27</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>36</td>
<td>36.0</td>
<td>64</td>
<td>64.0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Cal$X^2 = 2.691$  Tab$X^2 = 5.99$  DF= 2  p. value 0.260

These current results are consistent with study results that indicated non-statistically significant differences in genotype distributions of patients with and without a family history for each person. There are no allelic frequencies and genotypes for TGFβ1 SNPs examined in placental tissue in first-trimester miscarriages. However, in three of the eight non-pregnant women with a history of RPL, the TGFβ1 gene was elevated. This is the result of the influence of genetics on the incidence of miscarriage in women.

4.3. DNA sequencing

Through the results samples obtained after sending to the gene bank DDBJ, analyze it and compare it with the normal genes VEGF, TGFβ1 are shown. The sequence of the nucleotide of the VEGF, TGFβ1 genes were known in 150 samples, 50 pregnant women samples and 100 miscarried women samples as 25 microliters of each sample of the PCR product with the Primers of the VEGF, TGFβ1 genes were sent to Macrogen in the South Korea and after obtaining the results all the results were compared directly with the nucleotide of the VEGF, TGF β1 genes available in the internet http://www.ddbj.nig.ac.jp by computer program http://www.mbio.ncsu.edu/bioedit/bioedit.html the results showed that there were points mutations in samples patients with the VEGF, TGFBI gene as it was found that there is more than one mutation in the samples patients this shows that the type and location of the mutations that have been found may lead to a different effect of the mutations. Some of these mutations lead to changes in the genetic codes.
Figure (1) PCR products sequencing

GG       TTGCTTGCCATTTCCCACTTTGAATCGGCGCGAGCTTGGAGGAATGTCATCTTCCCC
60       GG

60       TTGCTTGCCATTTCCCACTTTGAATCGGCGCGAGCTTGGAGGAATGTCATCTTCCCC
60       GC

CC       TTGCTTGCCATTTCCCACTTTGAATCGGCGCGAGCTTGGAGGAATGTCATCTTCCCC
60       CC

120      GG       AAATCAGTGTGAATTTTGAAACCAGAAGAGAGGAAGGATGAAAGGATGAGCAGGAGGCTCCAGAG
60       GG

60       AAATCAGTGTGAATTTTGAAACCAGAAGAGAGGAAGGATGAAAGGATGAGCAGGAGGCTCCAGAG
60       GC

60       AAATCAGTGTGAATTTTGAAACCAGAAGAGAGGAAGGATGAAAGGATGAGCAGGAGGCTCCAGAG
60       CC

120      GC       AGAAGTCGAGGAAGAGAGAGACGGGGTCAGAGAGAGCGCGCGTGCGAGCAGCGAAA
60       GC

60       AGAAGTCGAGGAAGAGAGAGACGGGGTCAGAGAGAGCGCGCGTGCGAGCAGCGAAA
60       CG

60       AGAAGTCGAGGAAGAGAGAGACGGGGTCAGAGAGAGCGCGCGTGCGAGCAGCGAAA
60       CG

120      CC       CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGCGGACAGTGGACGCGGCGGCGA
60       CC

60       CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGCGGACAGTGGACGCGGCGGCGA
60       CG

60       CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGCGGACAGTGGACGCGGCGGCGA
60       CG

180      GG       CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGAGGACAGACAGACAGACACCG
60       GG

60       CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGAGGACAGACAGACAGACACCG
60       GC

60       CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGAGGACAGACAGACAGACACCG
60       CG

240      GG       GGACAGGGGCAAATTGAGTACCTGCTTTTGGGAGTACCCCGGAGCGCGCCGCGGTGAGG
60       GG

60       GGACAGGGGCAAATTGAGTACCTGCTTTTGGGAGTACCCCGGAGCGCGCCGCGGTGAGG
60       GC

60       GGACAGGGGCAAATTGAGTACCTGCTTTTGGGAGTACCCCGGAGCGCGCCGCGGTGAGG
60       CG

240      GC       GCGTCGCACTGAAACTTTTCGTCCAAC
60       GC

60       GCGTCGCACTGAAACTTTTCGTCCAAC
60       CG

60       GCGTCGCACTGAAACTTTTCGTCCAAC
60       CG

300      GG       CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGAGGACAGACAGACAGACACCG
60       GG

60       CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGAGGACAGACAGACAGACACCG
60       GC

60       CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGAGGACAGACAGACAGACACCG
60       CG

360      GG       CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGCGGACAGTGGACGCGGCGGCGA
60       GG

60       CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGCGGACAGTGGACGCGGCGGCGA
60       GC

60       CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGCGGACAGTGGACGCGGCGGCGA
60       CG

420      GG       GCCGCCGCGGCGCGGCGGAAGGGCGAGGGCGGGGCGGGGGGTTGGGGTCGGGCGCTCGG
60       GG

60       GCCGCCGCGGCGGCGGCGGAAGGGCGAGGGCGGGGCGGGGGGTTGGGGTCGGGCGCTCGG
60       GC

60       GCCGCCGCGGCGGCGGCGGAAGGGCGAGGGCGGGGCGGGGGGTTGGGGTCGGGCGCTCGG
60       CG

469      GG       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       GG

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       GC

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      GC       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       GC

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      CC       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CC

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      CG       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      CC       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CC

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      CG       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      CC       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

469      CG       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG

60       GCCGTCGCACTGAAACTTTTTCGTCGTCACCTTTGGGCTGCTTCCGAGGAGGGGCTGCTGCTG
60       CG
5. Conclusion

1. Polymorphisms in the VEGF gene may not have any effect on the prevalence of recurrent miscarriage in women in the Thi-Qar community, and this conclusion requires an extensive study to prove it.

2. In certain Thi-Qar groups, there was a correlation between the occurrence of recurrent miscarriages and TGFβ1 gene polymorphisms.
6. References


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