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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 aged37- 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 *TGFB1* genes have no impact on the prevalence of RM in Thi-Qar communities.

Key words: Recurrent Miscarriage, *VEGF* gene, *TGF- β 1* 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 of 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 from17 - 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 *TGF β 1* 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.

Product Name	Primer	Oligonucleotide sequence (5′-3′)
VEGF [10]	Forward primer	5'TTGCTTGCCATTCCCCACTTGA -3'
	Reverse primer	5'-CCGAAGCGAGAACAGCCCAGAA-3'
TGF β 1[11]	Forward primer	5'- GCAGTTGGCGAGAACAGTTG-3'
	Reverse primer	5'- CCAGAACGGAAGGAGAGTCAG-3'

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

Locus	Product size (bp)	Tm (degree)	Cycle conditions
VEGF	469 bp	60°C	38
TGFB1	597 bp	62°C	38

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

Age Groups in years	Patients		Control		Total	
	No.	%	No.	%	No.	%
17 - 26	38	38.0	25	50.0	63	42.0
27 - 36	42	42.0	17	34.0	59	39.33
37 - 45	20	20.0	8	16.0	28	18.67
Total	100	66.67	50	33.33	150	100
CalX^2= 2.923	**TabX^2= 5.99**		**DF= 2**		**p. value 0.232**	
Age means	Patients	29.2 ± 7.35			p. value 0.323	
	Control	27.9 ± 6.94				

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

Level Education	Patients		Control		Total	
	No.	%	No.	%	No.	%
Literacy	40	40.0	7	14.0	47	31.33
Primary	32	32.0	26	52.0	58	38.67
Middle	18	18.0	14	28.0	32	21.33
University	10	10.0	3	6.0	13	8.67
Total	100	66.67	50	33.33	150	100
$CalX^2$= 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

Family History	Family History		Non-family History		Total	
	No.	%	No.	%	No.	%
Patients	36	36.0	64	64.0	100	66.67
Control	5	10	45	90.0	50	33.33
Total	41	27.33	109	72.67	150	100
$CalX^2$= 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**

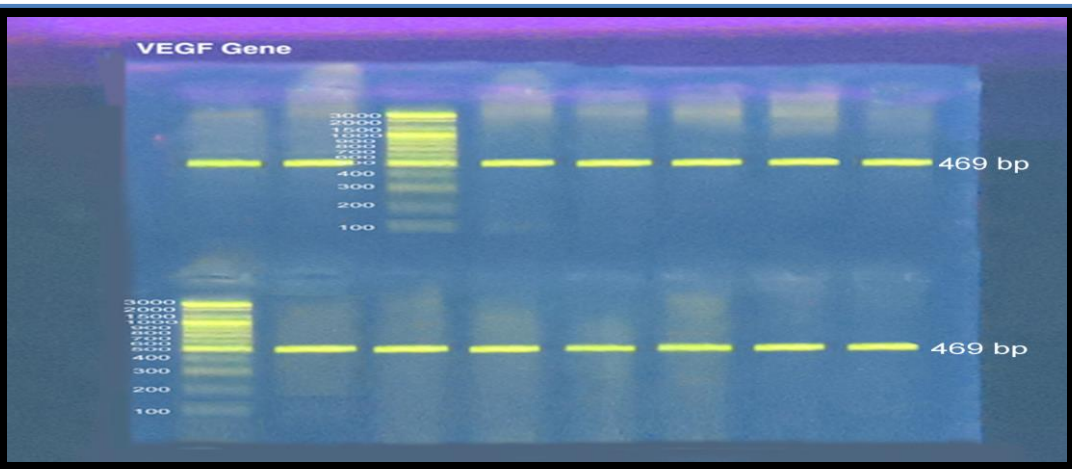
The current results noted the high miscarriaged women in those infected with other disease 58%, while the lowest miscarriaged women in those non other disease 42%. In other hand, the high women in the control group in those non other disease 86%, and the lowest in those non disease 14%, the results also, 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

Other diseases	Other diseases		Non-other diseases		Total	
	No.	%	No.	%	No.	%
Patients	58	58.0	42	42.0	100	66.67
Control	7	14.0	43	86.0	50	33.33
Total	65	43.33	85	56.67	150	100
$CalX^2$= 42.014	$TabX^2$= 3.84	DF= 1	p. value < 0.01**			

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 ^{mt} "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.

VEGF **Gene**	**Genotype**	**Patients No. 100**		**Control No. 50**		**CalX^2 p. value**	**OR 95%CI**
		No.	**%**	**No.**	**%**		
G182C	C/C	12	12.0	9	18.0	0.215	Non-OR
	G/C	45	45.0	25	50.0		
	G/G	43	43.0	16	32.0		
	Allele	**Patients**		**Control**		**CalX^2**	**OR 95%CI**
	C	57	39.31	34	35.79	0.661	1.13 (0.64 - 2.0)
	G	88	60.69	41	64.21		

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 [19]. and with others who said that no correlation has been observed across several populations. However, these results differ from those of [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 classis of genotypes and age of patients, at p. value < 0.05.

Table 8: Association between *VEGF* genotypes and age of miscarriaged

***VEGF* genotype**	**CC**		**GC**		**GG**	
	No.	**%**	**No.**	**%**	**No.**	**%**
17 – 26	2	2.0	17	17.0	19	19.0
27 – 36	4	4.0	21	21.0	17	17.0
37 – 45	6	6.0	7	7.0	7	7.0
Total	12	12.0	45	45.0	43	43.0
CalX^2= 8.52	**TabX^2= 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] .

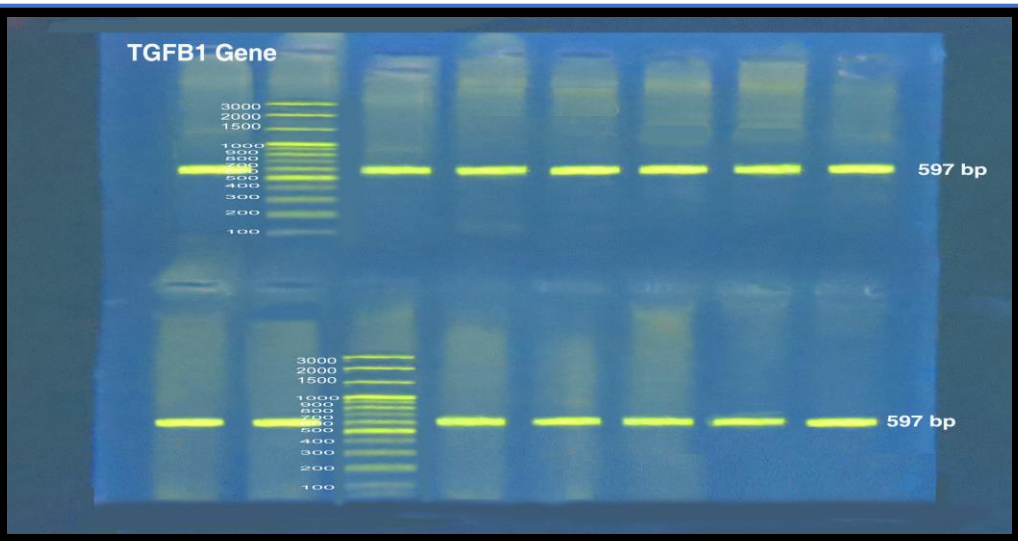
4.2.3. *VEGF* Genotypes and Family History in Miscarried Women

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

Family History ***VEGF* genotype**	**Family History**		**Non-family history**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
CC	5	41.67	7	58.33	12	12.0
GC	19	42.22	26	57.78	45	45.0
GG	12	27.91	31	72.09	43	43.0
Total	36	36.0	64	64.0	100	100
CalX^2= 5.585	**TabX^2= 5.99**		**DF= 2**		**p. value 0.061**	

Prvious 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].



- **Figure (2):** Product size for ***TGFB1*** 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"

4.2.4. Frequency of genotypes for the *GTF-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 miscarriaged 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 miscarriaged 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.

***TGF-β1* Gene**	**Genotype**	**Patients No. 100**		**Control No. 50**		**CalX^2 p. value**	**OR 95%CI**
		No.	**%**	**No.**	**%**		
C488T	C/C	30	30.0	16	32.0	0.023*	Non-OR
	C/T	43	43.0	28	56.0		
	T/T	27	27.0	6	12.0		

	Allele	Patients		Control		CalX^2	OR 95%CI
	C	73	51.05	44	56.41	0.478	0.818 (0.46 - 1.4)
	T	70	48.95	34	43.59		

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

***TGF- β1*genotype**	**CC**		**CT**		**TT**	
	No.	**%**	**No.**	**%**	**No.**	**%**
17 – 26	12	12.0	19	19.0	7	7.0
27 – 36	12	12.0	16	16.0	14	14.0
37 – 45	6	6.0	8	8.0	6	6.0
Total	30	30.0	43	43.0	27	27.0
CalX^2= 2.49	**TabX^2= 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

Family History	Family History		Non- family history		Total	
***GTF β1* genotype**	**No.**	**%**	**No.**	**%**	**No.**	**%**
CC	9	30.0	21	70.0	30	30.0
CT	16	37.21	27	62.79	43	43.0
TT	11	40.74	16	59.26	27	27.0
Total	36	36.0	64	64.0	100	100
CalX^2= 2.691 **TabX^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. [19], 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 [26]. 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*, *TGFB1* 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**.**

VEGF. 182.G>C SNP Alignments

```
 60        GG      TTGCTTGCCATTCCCCACTTGAATCGGGCCGACGGCTTGGGGAGATTGCTCTACTTCCCC
 60        GC      TTGCTTGCCATTCCCCACTTGAATCGGGCCGACGGCTTGGGGAGATTGCTCTACTTCCCC
 60        CC      TTGCTTGCCATTCCCCACTTGAATCGGGCCGACGGCTTGGGGAGATTGCTCTACTTCCCC
                   ************************************************************

120        GG      AAATCACTGTGGATTTTGGAAACCAGCAGAAAGAGGAAAGAGGTAGCAAGAGCTCCAGAG
120        GC      AAATCACTGTGGATTTTGGAAACCAGCAGAAAGAGGAAAGAGGTAGCAAGAGCTCCAGAG
120        CC      AAATCACTGTGGATTTTGGAAACCAGCAGAAAGAGGAAAGAGGTAGCAAGAGCTCCAGAG
                   ************************************************************

180        GG      AGAAGTCGAGGAAGAGAGAGACGGGGTCAGAGAGAGCGCGCGGGCGTGCGAGCAGCGAAA
180        GC      AGAAGTCGAGGAAGAGAGAGACGGGGTCAGAGAGAGCGCGCGGGCGTGCGAGCAGCGAAA
180        CC      AGAAGTCGAGGAAGAGAGAGACGGGGTCAGAGAGAGCGCGCGGGCGTGCGAGCAGCGAAA
                   ************************************************************

240        GG      GGGACAGGGGCAAAGTGAGTGACCTGCTTTTGGGGGTGACCGCCGGAGCGCGGCGTGAGC
240        GC      GSGACAGGGGCAAAGTGAGTGACCTGCTTTTGGGGGTGACCGCCGGAGCGCGGCGTGAGC
240        CC      GCGACAGGGGCAAAGTGAGTGACCTGCTTTTGGGGGTGACCGCCGGAGCGCGGCGTGAGC
                   * ************************************************************

300        GG      CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGACGGACAGACAGACAGACACCG
300        GC      CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGACGGACAGACAGACAGACACCG
300        CC      CCTCCCCCTTGGGATCCCGCAGCTGACCAGTCGCGCTGACGGACAGACAGACAGACACCG
                   ************************************************************

360        GG      CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGGCGGACAGTGGACGCGGCGGCGA
360        GC      CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGGCGGACAGTGGACGCGGCGGCGA
360        CC      CCCCCAGCCCCAGCTACCACCTCCTCCCCGGCCGGCGGCGGACAGTGGACGCGGCGGCGA
                   ************************************************************

420        GG      GCCGCGGGCAGGGGCCGGAGCCCGCGCCCGGAGGCGGGGTGGAGGGGGTCGGGGCTCGCG
420        GC      GCCGCGGGCAGGGGCCGGAGCCCGCGCCCGGAGGCGGGGTGGAGGGGGTCGGGGCTCGCG
420        CC      GCCGCGGGCAGGGGCCGGAGCCCGCGCCCGGAGGCGGGGTGGAGGGGGTCGGGGCTCGCG
                   ************************************************************

                469  GG      GCGTCGCACTGAAACTTTTCGTCCAACTTCTGGGCTGTTCTCGCTTCGG
                469  GC      GCGTCGCACTGAAACTTTTCGTCCAACTTCTGGGCTGTTCTCGCTTCGG
                469  CC      GCGTCGCACTGAAACTTTTCGTCCAACTTCTGGGCTGTTCTCGCTTCGG
                             ************************************************
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***TGF β1*. 488.C>T SNP Alignments**

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CC      GCAGTTGGCGAGAACAGTTGGCACGGGCTTTCGTGGGTGGTGGGCCGCAGCTGCTGCATG     60
CT      GCAGTTGGCGAGAACAGTTGGCACGGGCTTTCGTGGGTGGTGGGCCGCAGCTGCTGCATG     60
TT      GCAGTTGGCGAGAACAGTTGGCACGGGCTTTCGTGGGTGGTGGGCCGCAGCTGCTGCATG     60
        ************************************************************

CC      GGGACACCATCTACAGTGGGGCCGACCGCTATCGCCTGCACACAGCTGCTGGTGGCACCG     120
CT      GGGACACCATCTACAGTGGGGCCGACCGCTATCGCCTGCACACAGCTGCTGGTGGCACCG     120
TT      GGGACACCATCTACAGTGGGGCCGACCGCTATCGCCTGCACACAGCTGCTGGTGGCACCG     120
        ************************************************************

CC      TGCACCTGGAGATCGGCCTGCTGCTCCGCAACTTCGACCGCTACGGCGTGGAGTGCTGAG     180
CT      TGCACCTGGAGATCGGCCTGCTGCTCCGCAACTTCGACCGCTACGGCGTGGAGTGCTGAG     180
TT      TGCACCTGGAGATCGGCCTGCTGCTCCGCAACTTCGACCGCTACGGCGTGGAGTGCTGAG     180
        ************************************************************

CC      GGACTCTGCCTCCAACGTCACCACCATCCACACCCCGGACACCCAGTGATGGGGGAGGAT     240
CT      GGACTCTGCCTCCAACGTCACCACCATCCACACCCCGGACACCCAGTGATGGGGGAGGAT     240
TT      GGACTCTGCCTCCAACGTCACCACCATCCACACCCCGGACACCCAGTGATGGGGGAGGAT     240
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CC      GGCACAGTGGTCAAGAGCACAGACTCTAGAGACTGTCAGAGCTGACCCCAGCTAAGGCAT     300
CT      GGCACAGTGGTCAAGAGCACAGACTCTAGAGACTGTCAGAGCTGACCCCAGCTAAGGCAT     300
TT      GGCACAGTGGTCAAGAGCACAGACTCTAGAGACTGTCAGAGCTGACCCCAGCTAAGGCAT     300
        ************************************************************

CC      GGCACCGCTTCTGTCCTTTCTAGGACCTCGGGGTCCCTCTGGGCCCAGTTTCCCTATCTG     360
CT      GGCACCGCTTCTGTCCTTTCTAGGACCTCGGGGTCCCTCTGGGCCCAGTTTCCCTATCTG     360
TT      GGCACCGCTTCTGTCCTTTCTAGGACCTCGGGGTCCCTCTGGGCCCAGTTTCCCTATCTG     360
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CC      TAAATTGGGGACAGTAAATGTATGGGGTCGCAGGGTGTTGAGTGACAGGAGGCTGCTTAG     420
CT      TAAATTGGGGACAGTAAATGTATGGGGTCGCAGGGTGTTGAGTGACAGGAGGCTGCTTAG     420
TT      TAAATTGGGGACAGTAAATGTATGGGGTCGCAGGGTGTTGAGTGACAGGAGGCTGCTTAG     420
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CC      CCACATGGGAGGTGCTCAGTAAAGGAGAGCAATTCTTACAGGTGTCTGCCTCCTGACCCT     480
CT      CCACATGGGAGGTGCTCAGTAAAGGAGAGCAATTCTTACAGGTGTCTGCCTCCTGACCCT     480
TT      CCACATGGGAGGTGCTCAGTAAAGGAGAGCAATTCTTACAGGTGTCTGCCTCCTGACCCT     480
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CC      TCCATCCCTCAGGTGTCCTGTTGCCCCCTCCTCCCACTGACACCCTCCGGAGGCCCCCAT     540
CT      TCCATCCYTCAGGTGTCCTGTTGCCCCCTCCTCCCACTGACACCCTCCGGAGGCCCCCAT     540
TT      TCCATCCTTCAGGTGTCCTGTTGCCCCCTCCTCCCACTGACACCCTCCGGAGGCCCCCAT     540
        ******* ****************************************************

CC      GTTGACAGACCCTCTTCTCCTACCTTGTTTCCCAGCCTGACTCTCCTTCCGTTCTGG 597
CT      GTTGACAGACCCTCTTCTCCTACCTTGTTTCCCAGCCTGACTCTCCTTCCGTTCTGG 597
TT      GTTGACAGACCCTCTTCTCCTACCTTGTTTCCCAGCCTGACTCTCCTTCCGTTCTGG 597
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Figure (2) PCR products sequencing

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.

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