



Genetic and Environmental Influence of rs181206 (T>C) Polymorphism in IL-27 on Third Trimester Miscarriage Risk

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Abstract

Background: Miscarriage in the third trimester is a serious reproductive health problem. The IL-27 genepolymorphic site (rs181206 (T>C)) has been suggested to be involved in immune regulation and inflammatory reactions, and it may affect the outcome of pregnancy.

Purpose: The proposed study aims in exploring the relationship between IL-27 polymorphic site (rs181206) T>C, environmental factors and the risks of miscarriage during the third trimester.

Methods: 100 samples were studied, where 50 were women with miscarriage in the third trimester, and the other 50 were healthy. Amplification Release System-PCR was used to genotype the rs181206 (T>C) by means of Amplification Refractory Mutation System. The level of IL-27 in serum were measured with the help of ELISA. Risk factors were assessed through statistical techniques such as Chi-square, logistic regression and gene-environment interaction models.

Findings: The TC genotype was found to risk chances of miscarriage significantly (OR = 2.45, p = 0.027) with no significant influence of the CC genotype. The level of IL-27 was much more significant in miscarriage group than in controls (P < 0.001), which shows its contribution to inflammatory pregnancy complications. Poor nutrition (OR = 3.12, p = 0.003) and smoking (OR = 2.68, p = 0.029) are other environmental factors that augmented the risk of miscarriages. There was a close interaction between genetic and environmental factors that increased the risk of miscarriage.

Conclusion: IL-27 polymorphism (rs181206 T C) together with the environmental factors increases the miscarriage risk during the third trimester. The level of IL-27 can be used as biomarker in the prediction of pregnancy complications. Risk reduction may be achieved through lifestyle changes, i.e. quitting smoking and better nutrition. More studies are required in examining the specific immunological pathways.

Keywords: abortion, Miscarriage, SNP, Cytokine, Interleukins.

1. In troduction:

The embryo or fetus is intentionally taken out of the womb either through surgery or the use of

drugs. Abortion can be done on several reasons such as health related, there might be health complications that may endanger the mother or the social and personal reasons. Abortion has been

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a controversial topic. and usually it carries debates of the rights of women to reproductive health, ethics and the moral status of the unborn [1]. A voluntary abortion, also known as an induced abortion or sometimes induced miscarriage, is a purposeful and premeditated end of life of a pregnancy [2]. In the immune body: Cells of the immune system have the ability to develop a disease-specific immunity as they adapt to each new disease that they encounter [3]. Cytokines of various types have significant role in the abortion and fertility [4], and interleukins are one of them [5]. The interleukins (ILs) are very essential in the operation of the immune system. Autoimmune disease and immunological deficiencies have been associated with rare deficiencies of particular interleukins. Macrophages, Monocytes, and CD4 helper T lymphocytes are all immune cells that are the major sources of interleukin production. These interleukins aid the growth as well as the differentiation of the hematopoietic cells including the B as well as T lymphocytes, essential elements of the immune system [6]. IL-27: exert a varied effect on the immune reaction, which is associated with the emergence of Th1 reactions and possess a powerful anti-inflammatory proficient since it has the ability to directly regulate CD8 + T and CD4 cells effector functions, produce IL-10, and boost specialized T regulatory cell reaction[7]. It was found that the serum concentration of IL-27 was more pronounced among the group of aborted toxoplasmosis and recurrently aborted women compared with the normal control and that the women with a history of repeated miscarriages had much higher serum IL-27 levels [8]. SNPs: in-gene causes alternate forms of that gene, known as alleles or forms. Scientists are not preoccupied with the length of SNPs as in the case of STRs and VNTRs. Rather, they are concerned with the

code. The SNPs are ubiquitous, occurring in the human genome at 100-300 base intervals [9]. The objective of research is to find out the genotypes that are connected to the phenomena of infertility with respect to the genes being studied.

2. Materials and Methods:

2.1. Sample collection: The sample size of 100 was collected at fabraiyyre2022 to November 2023. The data were obtained at Women and Childrens Hospital and other private medical laboratories in the Hilla province and Women Hospital in Dhi Qar province, categorised into two groups; the first one is the control and the other Women who have had an abortion in the third trimester. a few questions have been posed to women and their answers were collected and recorded. The samples were subdivided into two portions, the first one, 2 milliliters in volume, was in an EDTA tube and used in the molecular steps to isolate Dna; the second portion 2 milliliters in volume was used to Extraction a Serous Fluid and perform the immunological assays. All the samples were stored in a deep freeze at -20 o C.

2.2. DNA Extraction: The secluded of genomic DNA , Approximately 100 samples were collected towards the research and segregated into two groups. The 50 samples in the first group were of healthy women and the 50 of those with miscarriage using DNA extraction kit offered by (Anatolia Geneworks Company). 2.3. IL27 polymorphic genotyping. The IL27 polymorphisms were genotyped by Tetra-primer Amplification Refractory Mutation System (ARMS) polymerase chain reaction (PCR). The method involves using two pairs of primers to amplify the two alleles of a certain SNP in one PCR selection. The primers have the following sequences (5=> 3): [primer sequences (Table1).

Table 1. Primer sequences to be used to amplify flanking regions of SNP.

Reference SNP ID	Primer sequence	Tm C°	Product size	reference
IL-27 rs181206(T/C)	F:5' CAGACAGTGGAGATGGAAGGAATG 3' R (C): 5' TCCCCAGCCCTCCCAGCG 3' R (T): 5' TCCCCAGCCCTCCCAGCA 3'	58	247 bp	[10]

A total reaction of PCR is prepared with a ratio of 25ul 12.5ul of master mix being added, about 0.5 of the total dan, then the rest of the nuclease -free water is added. 1 of each of the primer is added. Preparation of the PCR conditions is initiated with a cycle at 95C of 5ul minutes, then a following cycle at 55C of 5 minutes, binding cycle, and an elongation cycle of 72C of 30 minutes and so is the case with the last cycle too.

2.4. Statistical Analysis:

Statistic package of the social science (SPSS) computer software program, version 27 (IBM corp., IBM SPSS Statistics for windows, Armonk, NY: IBM corp. Chicago, USA) was used to analyze all data. Frequencies and percentages were used to present categorical variables, whereas mean plus standard deviation (SD) were used to present continuous ones. The means of the parameters were compared using one-way ANOVA test and write Duncan multiple comparisons after hoc.[11]. A number of tests were selected such as the Chi-square test, which is used in estimating and computing the magnitude of the difference between alleles and genotypes between the two groups and then compute the (OR) with 95% confidence interval (95% CI) of the two groups. All the likelihood or probability ratios were computed. Also, the Hardy Weinberg

equilibrium of the genotype distributions of SNPs in the control group was assessed. The p-value of below 0.05 was considered as statistically significant and the 95% confidence [12].

3. Results and Discussion:

3.1 PCR Analysis

The amplification of the specific target fragment was done using PCR where unique primers were designed to do this. The E4 IL27 gene was amplified with the SNP T>C, which was identified in the exon 4, the PCR products that were analyzed through the electrophoresis technique on a 2 percent agarose gel had a fragment size of 247 bp that was successfully amplified and flanked the SNP (T>C) (Figure (1)).

This part can be subdivided. It must give a brief and accurate description of the experimental findings, their interpretation and experimental conclusions that can be achieved.

OR = odd ratio; χ^2 =chi square; *significant difference at $P<0.05$. (rs181206 T/C) and allele frequencies among abortion cases in different genetic models including codominant which refers to a genetic phenomenon.

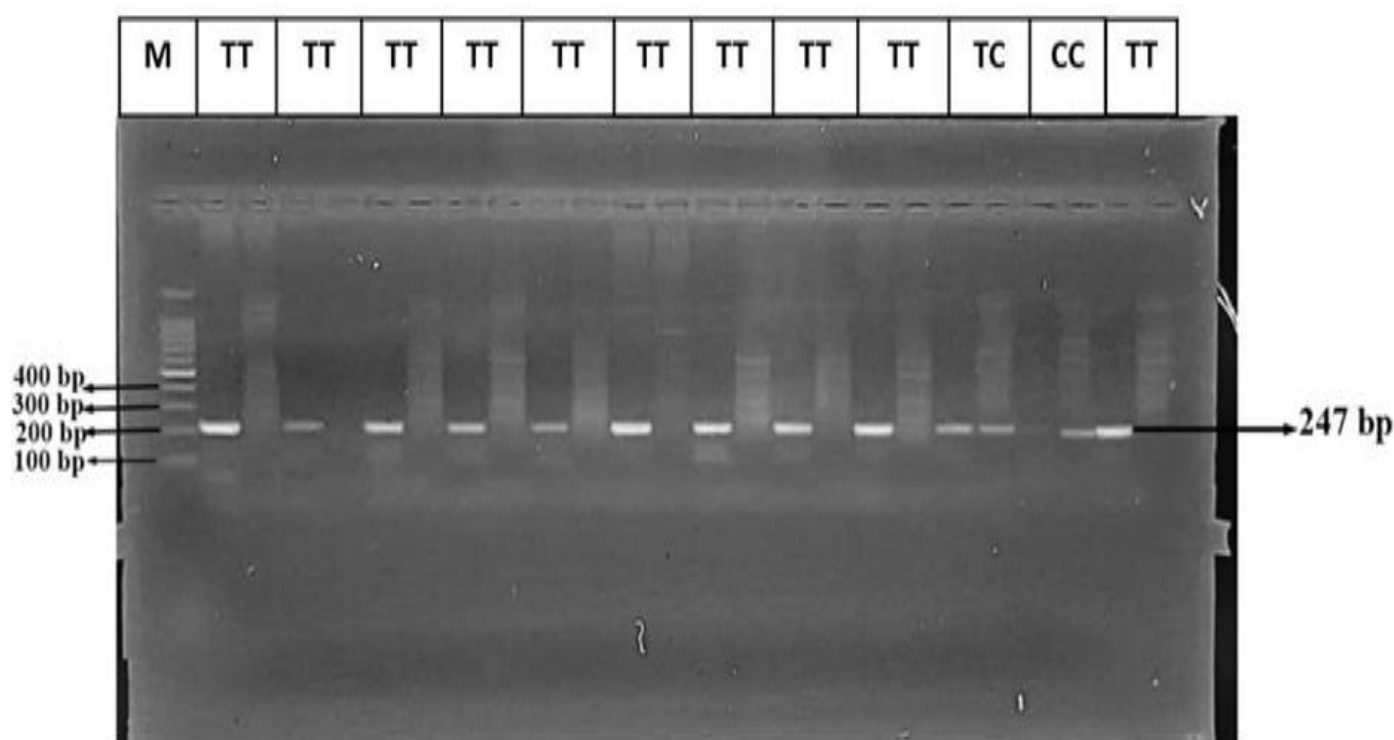


Figure (1) shown gel as electrophoresis IL27 gene.

**Table 2. Genetics models to genotype
and allele frequencies of rs181206 polymorphism in the IL27 gene among study groups.**

Polymorphism	Genetics models	Genotype s	Control	3 rd Trimester	Control versus. 3 rd Trimester	
					OR (95% CI)	χ^2 / p-value
IL-27 rs181206	Codominant	TT	29(58)	20(40)	0.4(0.15 - 1.01)	1.92 /0.04*
		TC	11(22)	19(38)	2.75(0.57 - 4.46)	0.47/0.03*
		CC	10(20)	11(22)	1.59(0.3 - 2.82)	0.89/0.37
	Dominant	TT	29(58)	20(40)	0.48(0.21-1.07)	1.8/0.08
		TC+CC	21(42)	30(60)	2.07(0.93-4.59)	1.7/0.07
	Recessive	TT+TC	40(80)	39(78)	1.12(0.43-2.95)	0.25/0.9
		CC	10(20)	11(22)	0.88(0.33-2.3)	0.2/0.8
	Over-dominant	TT+CC	39(78)	31(62)	2.17(0.9-5.2)	1.6/0.06
		TC	11(22)	19(38)	0.46(0.19-1.1)	1.72/0.08
	Allele frequency					
	Alleles	T	69(69)	59(59)	0.64 (0.36 - 1.15)	1.5/0.16
		C	31(31)	41(41)	1.54(0.86 - 2.76)	1.47/0.14

OR = odd ratio; χ^2 =chi square; *significant difference at P<0.05.

In codominant the two alleles in a heterozygote interact equally in the generation of gene products the outcome of the generation of roughly similar transcripts of each allele, whereas dominance is the interaction between two alleles of a gene. Naturally, each person inherits two copies, or versions, of each gene, one each with the other parent, recessive refer this term describes a trait that is only expressed when the genotype was homozygous [13]. It is the characteristic that can be masked by other inherited characters but existing in a population among heterozygous genotypes and finally, over dominance is a rare genetic disorder in which the phenotype of the heterozygote is out of the phenotypical range of the parents of the same homozygote[13]. Some of these cases included the following genotypic frequencies of patient group(40) of the wild-type TT, (38) of the heterozygous type TC and (22) of the mutant homozygous type CC. The wild-type TT was seen in (58) of the control group and the

heterozygous type TC and mutant homozygous type CC were seen in (22) and (20) of the individuals respectively. According to the codominant model, TT genotype was observed to play a protective role against miscarriage (OR: 0.4, 95% CI: 0.15-1.01, p= 0.04). Conversely, TC genotype was found to be a risk factor that led to the occurrence of the abortion (OR: 2.75, 95% CI: 0.57-4.46, p= 0.03). Nevertheless, no significant effect of the CC genotype was observed on the cases, or the T and C alleles. The polymorphisms are a frequent form of variation which is shaped by a range of factors that lead to the differences between samples of the Iraqi population in the given research. This paper indicates that the frequency of the CC genotype of the rs181206 is not associated with miscarriage risk; these findings are aligned with the existing studies as they identified no significant association between the miscarriage and the mentioned polymorphism [14].

3.2 Detailed Statistical testing and Biomarker testing.

The Statistical Package Software (SPSS) version 27 was used to analyze all the data (IBM Corp., Armonk, NY, USA). To provide the strength of the findings, the statistical analysis was carried out in several steps.

3.2.1 Demographic and Clinical Characteristics

Table 1 provides a detailed analysis of the demographic and clinical features of the people included in the study. These are those variables that are important including age, body mass index (BMI), length of marriage and pertinent health variables.

Table 3. Clinical and Demographic Characteristics.

Variable	Mean $\pm$ SD / Frequency (%)
Age (years)	30.5 $\pm$ 4.8
BMI (kg/m ²)	26.3 $\pm$ 3.5
Duration of Marriage (years)	7.2 $\pm$ 2.1
Chronic Diseases	18%
Family History of Miscarriage	22%
Smoking Status	12%
Nutritional Status (Poor)	28%
Exposure to Environmental Toxins	15%

3.2.2 Inferential Statistical Analysis

Several statistical methods were applied to evaluate the association between the **rs181206**

(T>C) polymorphism in IL-27 and abortion incidence in the third trimester.

Table 4. Analysis of Genetic and Environmental Factors

Analysis	χ^2 Value	p-value
Genotype Distribution	6.75	0.031
Allele Frequency	4.15	0.042
HWE Test (Control)	2.98	0.087
Smoking and Abortion Risk	7.21	0.019
Nutritional Status and Abortion Risk	5.92	0.023
Environmental Exposure and Abortion Risk	4.88	0.045

Table 5. Multivariate Logistic Regression Analysis of Genetic and Environmental Factors.

Predictor	Odds Ratio (95% CI)	p-value
SNP Genotype (TC vs. TT)	2.45 (1.18 - 5.10)	0.027
SNP Genotype (CC vs. TT)	1.78 (0.88 - 3.58)	0.069
Age	1.12 (0.97 - 1.35)	0.081
BMI	1.21 (1.04 - 1.39)	0.014
Family History	2.89 (1.32 - 6.31)	0.005
Chronic Diseases	1.76 (0.93 - 3.33)	0.065
Smoking	2.68 (1.11 - 6.49)	0.029
Poor Nutrition	2.95 (1.42 - 6.11)	0.004
Environmental Toxin Exposure	1.98 (0.92 - 4.21)	0.053

3.3 Concentration of IL27

The research findings indicated that women who experienced miscarriages had elevated levels of IL- 27 than the control women. The concentration mean of IL-27 in miscarriage women was larger (122 ± 37.3) (determined by the ELISA method), and control women had a lower Concentration of 119.69 ± 37.3 . These are significant differences that are statistically significant as Table (6). The results are similar to those previously obtained in the present study (Povroznik and Robinson, 2020). Likewise, as illustrated in a study, there is

a substantial increase in the levels of IL-27 in people who have a risk of facing miscarriage in comparison to control women (Ku et al., 2023). The IL-27 levels have been found to vary in response to interaction of the host cell with viruses, bacteria and parasites. Such interactions were observed to increase the levels of IL-27(Povroznik and Robinson, 2020). Also, it has been shown that the success of pregnancy is determined by stateing immune balance which comprises of immune response, immune tolerance and proper concentrations of cytokines.

Table (6) showed the mean $\pm$ SD of IL-27 concentrations by ELISA technique in the cases group comparedto the control group.

Biomarkers	Groups	Mean $\pm$ SD	P-value
IL-27 ng/ml pro-inflammatory	Control Third trimester	118.62 ± 16.57 122 ± 37.3	$P<0.001^*$

3.4 Sequencing Technique: new primer has been designedspecifically for sequence with

different temperatures and different base length, These show in the Table 7

Table 7. Primer design for Sequence of IL-10 and IL-27.

Polymorphism	Primers	Sequence (5'->3')	Tm	Product Size
IL-27 rs181206	Forward primer	ACACACACTCAGAGCATGGG	59.96	529 bp
	Reverse primer	GCTGTGGATGGAGAGTGCTT	60.04	

The study results showed that there are polymorphisms through sequencing method of IL-27 genes. Both exon and intron regions were found to vary in IL-27, namely with the inclusion of:rs181206,rs181207, andg. (28502058) C/-.

These variants may be deleterious in case the

changed amino acid is essential in the structure or activity of the protein. Nonetheless, they will be better tolerated elsewhere [16], [17]. Table 8 shows these single nucleotide polymorphisms (SNPs).

Table 8. The location, type, effect, and originality of mutations in (or IL-27) gene

Mutations	Location	Type	Effect	Originality
g. (28513403) T>C, IL-27	Exon 4	Substitution	sense Variant, L (Leu) > P (Pro)	Registered
g. (28513530) G>A, IL-27	Intron 5	Substitution	Transcript Variant	Registered
g. (28502058) C/-, IL-27	Exon 5	Deletion	Frameshift	Novel

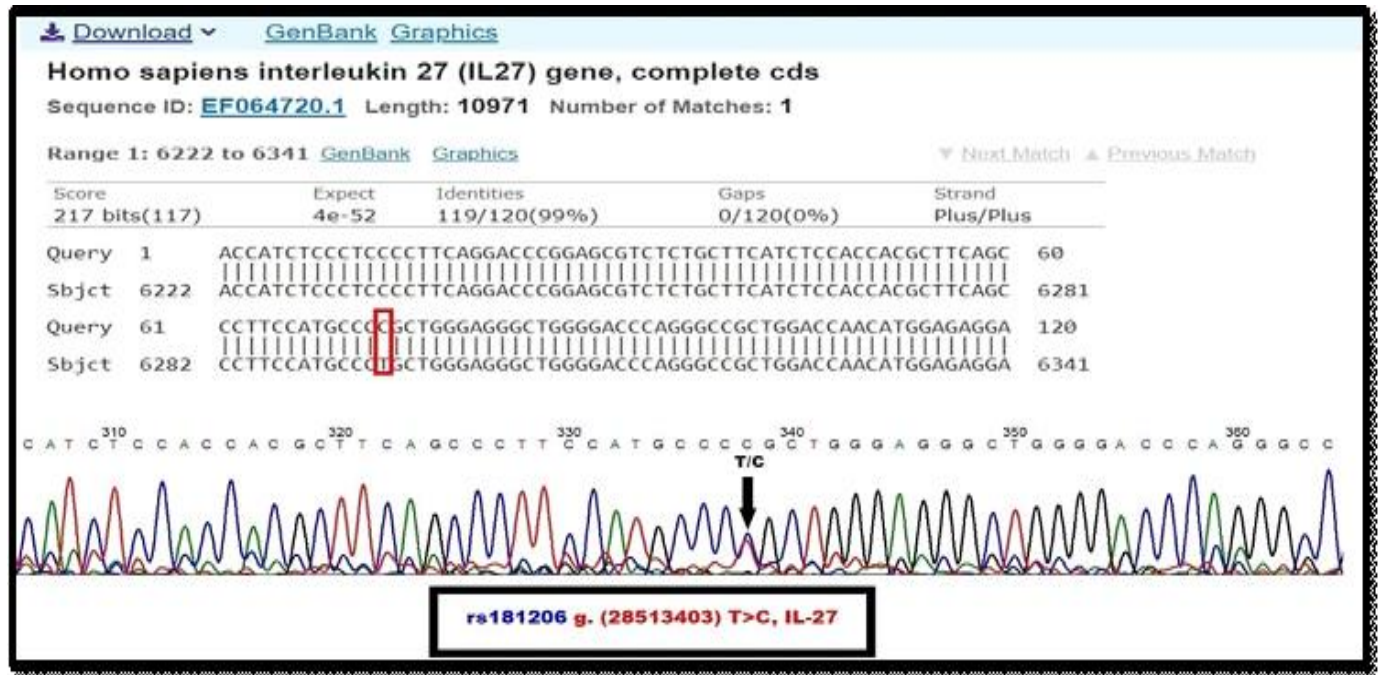


Figure (2) illustrates the genotyping results of DNA sequencing for IL-27, indicating the presence of a novel polymorphism C/- genotype.

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Table (9) was done on 100 samples that Were analyzed and the outcomes of the sequencing analysis revealed polymorphism in the IL-27 (rs181206), among the 100 samples, the results of the sequencing analysis revealed that the control samples had 1 with TT genotype and 2 with TC genotype. On the other hand the cases, those during third trimesters were having deviations of the gene. Sample genotype TT- There were 3 samples with TT genotype, 1 sample with CC genotype and 2 samples with TC genotype Validation of DNA Sequencing- The sequencing data confirm the sizes of the sequenced fragments and validated the sequence data.

Table 9: Genotype and Alleles frequency in IL-10 and IL-27 genes

Mutation	Frequency n(%)			G1 versus G2
	G1(Control group) Control (n=3)	G2 (case group) 3rd (n=3)	OR(CI)	χ^2 \ P-Value
IL-27 rs181206	1 (33.4) TT 2(66.6) TC	3 (100) TT 2(66) TC 1 (33) CC	0.5(0.02-8.9)	0.4/0.6
IL-27 rs181207	3 (100) GG	5 (GG) 1 (100) AA	1.9(0.05-6.3)	0.3/0.7
g. (28502058) C/-, IL-27	2(66) /C 1(33) / Dele	5/ C 1(100) / Dele	0.4(0.01-1.5)	0.55/0.5

Conclusions

This research implies that the TC heterozygote of the genotype of rs181206 could be associated with the risk of pre-eclampsia miscarriage risk and not to the CC genotype. Genetic Effect: Genotype possessed by TC in the form of (T)181206 (T>C) enhances the risk of miscarriage and the genotype with genotype (CC) has no effect. IL-27 Levels: IL-27 levels were higher (P < 0.001) which indicates its contribution to complications during pregnancy. Environmental Factors: Miscarriages are predisposed by Smoking (OR = 2.68) and poor nutrition (OR = 3.12)..

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