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An immunological and molecular study of Interleukin 4 in patients with type 1 and 2 diabetes

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ABSTRACT

Background: Type 1 diabetes (T1D) is a chronic autoimmune disease. It results in the destruction of pancreatic beta cells by immune cells. Type 2 diabetes (T2D) is a heterogeneous metabolic disorder. Hyperglycemia occurs due to insulin deficiency or impaired secretion. It is also a chronic inflammatory disease. Interleukin 4 (IL-4) plays an anti-inflammatory role and is produced by some immune cells. Studies suggest that genetic variations in the IL-4-related gene (590 C>T) may be genetic risk factors for diabetes.

Objective: To evaluate the concentration of interleukin-4 and investigate the polymorphism in the IL-4 gene region (IL-4 590 C>T) and their association with type 1 and type 2 diabetes.

Methods: Blood samples were drawn from 30 male patients with type 2 diabetes and 24 non-diabetic controls. Using sandwich ELISA technique, interleukin-4 concentrations were evaluated. The polymorphism of the mutant gene for interleukin-4 was detected using Amplification-refractory mutation system analysis of point mutations (ARMS) technique.

Results: The concentration of IL-4 decreased significantly in T1D patients compared to the control sample. The statistical significance value was P=0.022. In T2D patients, the decrease in IL-4 was not significant compared to the control sample, with a P=0.52. The differences in IL-4 concentration between T1D and T2D were significant. The TT and CT genotypes appeared to be a protective factor against the risk of developing T1D and T2D. In contrast, the CC and CT

genotypes were considered a risk factor that induces the development of T1D and T2D. **Conclusion:** The genetic makeup of the IL-4 (590 C>T) mutant gene patterns may affect the decreased IL-4 concentration in T1D and T2D diabetic patients.

Keywords: Type 1 and 2 diabetes, Interleukin-4, ARMS

INTRODUCTION

Type 1 Diabetes (T1D) is a chronic autoimmune disease characterized by the progressive destruction of pancreatic beta cells (β cells) by T helper (1Th) cells and others [1]. Type 2 Diabetes (T2D) is a heterogeneous metabolic disorder resulting from hyperglycemia resulting from insulin deficiency, insulin secretion deficiency, or both [2]. It is considered a chronic inflammatory disease. Stimuli, such as lethal genetic metabolic programming, overnutrition, or aging, can increase the levels of expressed cytokines [3]. Interleukin 4 (IL-4) is one of the cytokines that has attracted the attention of researchers as an anti-inflammatory. It is released by immune cells such as T helper cells, mast cells, and others [4]. Because the cause of the disease is immune, current treatments are primarily aimed at replacing exogenous insulin. This shows the importance of immunotherapy to improve clinical outcomes and reduce the disease. Accordingly, genetic studies have indicated the role played by cytokines in causing T1D. Considering that some of them are a major driver of inflammation and some of them play a role in controlling inflammation by destroying beta cells [3]. It was suggested that genetic variation in the mutant gene region (IL-4 590 C>T) is considered one of the genetic risk factors in autoimmune diseases, especially single nucleotide polymorphisms (SNP) [5]. Studies have shown that the gene responsible for encoding IL-4 is located on the long arm of chromosome 5 at the 5q31 site. It is 0.9 kb long and contains 4 exons [6]. IL-4 was discovered in the form of a polypeptide consisting of 129 amino acids. It plays an important role in regulating gene expression, cell proliferation, programmed cell death, and differentiation in many blood-forming cells. Several studies have shown that polymorphisms in the 590 regions can affect IL-4 production [7].

This study aimed to evaluate the concentration of interleukin-4. As well as to investigate the polymorphism in the IL-4 gene region (IL-4 590 C>T) and their relationship with type 1 and type 2 diabetes patients.

MATERIALS AND METHODS

Blood samples were collected from 30 people with T1D diabetes aged 3 to 30 years. Blood samples were collected from 30 people with T2D diabetes aged 30 to 68 years. In addition to control samples of 24 people without diabetes. The samples were collected randomly from Al-Shifa Teaching Hospital, Al-Mawain Teaching Hospital and Al-Faihaa Teaching Hospital in Basra Governorate - Iraq, for the period from January 25 to late July 2022. 5 ml of blood was drawn from each person in the study and divided into 3 ml in tubes that did not contain an anticoagulant to extract blood serum from each sample, which was later used to evaluate IL-4 concentrations in all study samples using the sandwich enzyme-linked immunosorbent assay (ELISA) technique. The diagnostic kit was used to perform this test from (BT LAB, China) according to the instructions contained in the diagnostic kit of the manufacturer.

As for the molecular genetic study, 2 ml of the remaining blood was placed in tubes containing an anticoagulant EDTA to be used in the process of extracting the genetic material DNA. This is done using the extraction kit from FAVORGEN BIOTECH CORP, Taiwan. The gene polymorphism of interleukin 4 (IL-) at the gene site -590 (C>T) was examined using the well-known Amplification-refractory mutation system analysis of point mutations (ARMS-PCR). The mixture Go Taq ® Green Master Mix (2X) was used according to the method of work from the American company Promega. Three primers were used to detect the mutant gene IL-4 590 (C>T) as they were designed according to [8] and are as shown in Table (1):

Table (1) Primers used to detect polymorphisms of the IL-4 gene

	Primer name	Nucleotide sequence
1	T allele	**5`GAATTTGTTAGTAATGCAGTCCTCC-3**
2	C allele	**5`ACACTAAACTTGGGAGAACATTGTC-3`**
3	Reverse	**5`GAATTTGTTAGTAATGCAGTCCTCC-3`**

The total volume of the reaction mixture was 25 µL and included 12.5 µL Go Taq ® Green Master Mix (2X) (1X concentration) and 2 µL of each primer (1.0 µM). In addition to 5 µL DNA template, the volume was made up to 25 µL with nuclease-free water. ARMS-PCR amplification was performed by placing the samples in a thermocycler. The thermocycler program was set to obtain

the reaction conditions. The amplification included a thermal cycle for 1 minute at 96°C, followed by 10 cycles of 95°C for 15 seconds, 65°C for 50 seconds, and 72°C for 40 seconds. Then, 20 cycles of 95° for 50 seconds, 59° for 50 seconds, 72° for 50 seconds. Then, the final extension included 72° for 7 minutes.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences ver.23 (SPSS). Statistical differences between means were compared using a t-test for the results of IL-4 concentrations for T1d diabetic patients with their standard samples, as they were normally distributed at a probability level of $P<0.05$. As for the results of IL-4 concentrations for T2D diabetic patients and the standard sample, since they were not normally distributed, the Mann-Whitney U test was used. The results of the alleles and genotypes under study were analyzed using the Hardy-Weinberg equilibrium. The frequencies of the genotypes and their alleles, the critical ratio Odd ratio (OD), and the confidence interval (CI) were analyzed using the Compare Ver.3.04 2 programs developed by J.H. Abramson.

RESULTS

Estimation of the level of concentration of interleukin-4 (Interleukin-4) in T1D

Table (2) shows a decrease in the concentration of IL-4 in the blood serum of T1D diabetic patients compared to the standard sample (control). When statistical analysis was performed, the results showed statistically significant differences in the concentration of IL-4 cytokines between T1D diabetic patients and standard samples at a probability level of $P\leq 0.05$.

Table (2) IL-4 concentration in the serum of T1D and standard samples

Cytokinesis	mean ± standard error ng/L samples for 30 T1D patients	95% CI		mean ± standard error ng/L for 10 standard samples	95% CI		Probability P
		Less value	Highest value		Less value	Highest value	

IL-4	112.57 ±9.018	0.00	208.21	166.22±28.26	0.00	344.88	**0.022**

When we take into consideration the age groups of the T1D diabetic patients sample, it was noted that there was a decrease in the arithmetic mean of IL-4 concentration in the age group less than or equal to 15 years (≥15) when compared with the age group of T1D diabetic patients greater than 15 years (>15) years. When conducting the statistical analysis, the results showed that there was no statistically significant difference in IL-4 concentration between people with T1D diabetes in the two age groups at a probability level of P≤ 0.05, Table (3).

Table (3) IL-4 concentration in the sera of the studied samples according to age groups

Age group (years)	Number of samples studied (30)	ELIZA Test Results Mean ± Standard Error ng/L	95% CI		Probability P
			Less value	Highest value	
(15≥)years	16	104.46±12.07	13.93	190.36	**0.345**
(15<)years	14	121.84±13.56	0.00	208.21	

Estimation of the level of concentration of interleukin-4 (Interleukin-4) in T2D patients

Table (4) shows a decrease in the arithmetic mean of the concentration of the cytokinetic IL-4 when compared with the standard sample. When conducting the statistical analysis, the results showed that there were no statistically significant differences in the concentration of the cytokinetic IL-4 between patients with T2D diabetes and the standard samples at the probability level of P≤ 0.05.

Table (4) IL-4 concentration in the sera of T2D samples and standard samples

Cytokinesis	mean ± standard error ng/L samples for 30 T2D patients	95% CI		mean ± standard error ng/L for 10 standard samples	95% CI		Probability P
		Less value	Highest value		Less value	Highest value	
IL-4	138.81 ±9.45	0.00	319.4	159.09±9.06	106.31	222.77	**0.52**

The age group of T2D patients less than or equal to 45 years (≥45) years had an increase in the arithmetic mean of IL-4 concentration compared to the second age group of T2D patients over 45 years (>45) years. It recorded a decrease compared to the first age group. The results of the statistical analysis showed that there was no statistically significant difference in IL-4 concentration between the two age groups at the probability level of P≤ 0.05 Table (5).

Table (5) IL-4 concentration in the sera of T2D patients' samples according to age groups

Age group (years)	Number of samples studied (30)	ELIZA Test Results Mean ± Standard Error ng/L	95% CI		Probability P
			Less value	Highest value	
(45≥)years	12	146.71±21.44	0.00	319.4	**0.626**
(45<)years	18	135.13±7.15	68.93	203.93	

Comparison of IL-4 concentration results in serum samples of T1D and T2D diabetic patients

Suppose the results of IL-4 concentration levels in serum samples of T1D and T2D diabetic patients are compared. In that case, we notice a slight difference in the arithmetic mean and standard error for patients with the two types of diabetes. T1D diabetes had the lowest

concentration compared to IL-4 concentration in T2D diabetic patients, which was the highest. The results of the statistical analysis showed a statistically significant difference in IL-4 concentration between people with T1D and T2D diabetes at a probability level of P≤ 0.05, Table (6).

Table (6) Comparison of IL-4 concentration results in serum samples of T1D and T2D diabetic patients.

Age group (years)	ELIZA Test Results Mean ± Standard Error ng/L	95% confidence limits		Probability P
		Less value	Highest value	
T1D	112.575±9.0186	0.00	208.21	0.025
T2D	138.813±9.4552	0.00	319.4	

Polymorphism of the IL-4 gene for the mutation site -590(C>T)

Polymorphism of the IL-4 gene for the mutation site -590(C>T) in T1D diabetic patients and the standard sample

Electrophoresis of the mutant gene IL-4 -590(C>T) was performed, which was amplified by ARMS-PCR. The presence of two alleles, t and the second allele, c, was found in T1D diabetic patients (30 samples) and standard samples (10 samples). Regarding the genotypes, the results revealed the presence of three genotypes: CT, CC and TT. In contrast, the TC and TT genotypes were the genotypes in the standard samples, as shown in Figures (1) and (2), respectively.

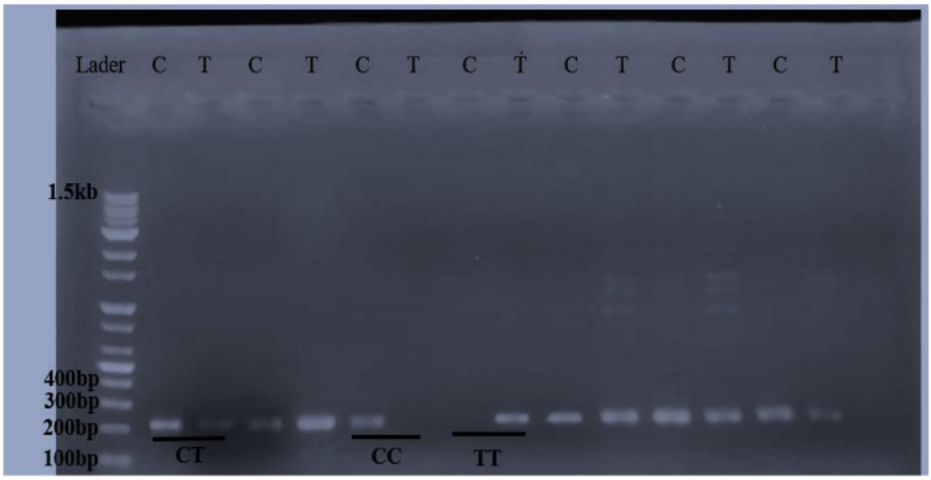

Figure (1) Electrophoresis of the mutant gene IL-4 -590 (C>T) showing the T and C alleles in patients with type 1 diabetes T1D.

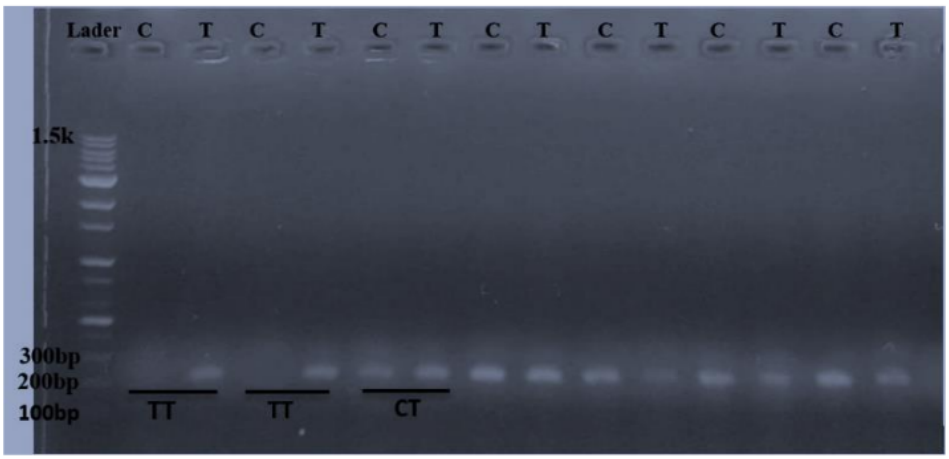

Figure (2) Electrophoresis of the mutant gene IL-4 -590 (C>T) showing the T and C alleles in the standard samples.

The results of the frequency distribution of the t and c alleles of the mutant gene IL-4 -590 showed, using the Hardy-Weinberg equilibrium law, the presence of a difference between the samples of T1D patients and the standard sample. In T1D diabetic patients, the t allele recorded a percentage of 53%, while the c allele recorded a percentage of 47%. On the other hand, the percentage of the t allele in the standard sample was 60%. As for the c allele, it recorded a percentage of 40%, as

shown in Table (7). On the other hand, the t and c alleles did not show a significant frequency in T1D diabetic patients and the standard sample using the Fishers test. As for the t allele, it did not show significant recurrence between T1D patients and the standard sample using Fishers test, as its critical ratio Odd Ratio (OR) recorded a value of 0.44 and the confidence interval (CI) under 95% ranged from 0.06 to 3.14. Its effect as a potential protective factor was 56%. As for the c allele, its critical ratio Odd Ratio (OR) for T1D patients and the standard sample was 0.88. The confidence interval (CI) under 95% ranged from 0.63-1.23. Its ratio as an allele causing the disease Ethiological faction (EF) was -0.1248. This value indicates the possibility of its protective association with the disease and not as a potential risk factor.

Table (7) Frequencies of the t and c alleles of the mutant gene IL-4 -590 (C>T) in T1D diabetic patients and the standard sample.

The gene	Allele	T1D patients(%)	Standard sample (%)	OR* (95%CI†)	P value
IL-4-590 (C>T)	T	53%	60%	0.44(CI=0.06 to 3.14)	**0.41**
	P.F‡	(1-0.44)*100=56%			
	C	47%	40%	0.88(CI=0.63 to 1.23)	
	EF §	- 0.12			

OR* = Odd ratio, † CI = Confidence Intervals, P.F‡ = Preventive Faction, § EF = Ethiological Faction.

The results of ARMS-PCR technology for the mutant gene IL-4-590 (C>T) and by using the Hardy-Weinberg equilibrium law during its genetic analysis showed the presence of three genotypes in T1D patients and the standard sample, which are CC, TT, and TC. The TT genotype reached 10% in T1D diabetic patients, and its percentage in the standard sample was 20%. Statistical analysis using Fisher's test showed no significant difference between T1D patients and the standard sample. Its value reached 0.41 at the probability level P<0.05, and the value of the critical ratio OR was 0.44. The confidence interval CI ranged from 0.06 to 3.14, and the value of the Preventive Faction (PF) was 56%. This indicates that this genotype may be a potential

protective factor rather than a risk factor. The highest prevalence of the genotypes was for the CT genotype in T1D patients, reaching 86.7%. In contrast, its prevalence in the control sample was 80%. The statistical analysis using Fisher's test showed no significant differences between T1D patients and the control sample at a probability level of P<0.05. Its value was 0.61, the critical ratio OR was 1.62, and the confidence interval CI ranged from 0.24 to 10.57. This makes the presence of this genotype a potential risk factor for T1D. Its prevalence as a protective factor PF is -0.625, which does not indicate an increased risk with the presence of the CT genotype. The statistical analysis using Fisher's test did not show any significant differences between the prevalence of the CC genotype in T1D patients and the control sample. Its percentage in them reached 3.3% and 0%, respectively, under the significance level of P<0.05. The critical ratio OR recorded a value of 1.06, the confidence interval was 0.04 to 28.29, and the value of its percentage as a causative factor of the disease EF is 6.35% Table (8).

Table (8) Frequencies of the genetic patterns of the mutant gene IL-4 -590 (C>T) in T1D diabetic patients and the standard sample.

The gene	Gene type	T1D patient%	Standard sample %	OR* 95% CI†	P value
IL-4-590 (C>T)	TT	10	20	0.44(0.06-3.14)	**0.41**
	PF‡		56%		
	CT	86.7	80	1.62(0.24-10-57)	**0.61**
	PF‡		-0.62		
	CC	3.3	0	1.06(0.04-28.29)	**0.96**
	EF§		6.35%		

OR* = Odd ratio, † CI = Confidence Intervals, P.F‡ = Preventive Faction, § EF = Ethiological Faction.

Results of the polymorphism of the IL-4 gene for the mutation site -590(C>T) IL-4 in T2D diabetic patients and the standard sample

The amplification process was carried out using ARMS technology for the mutant gene-590(C>T) IL-4 for T2D diabetic patients' samples (30 samples) and the standard sample (10). The electrophoresis process was carried out, and its results revealed the presence of two alleles, t and

the second allele, c. The results showed the presence of three genotypes, CT, TT and CC, in T2D diabetic patients. In contrast, the standard sample showed the presence of the CT genotype only, Figure (3) and (4).

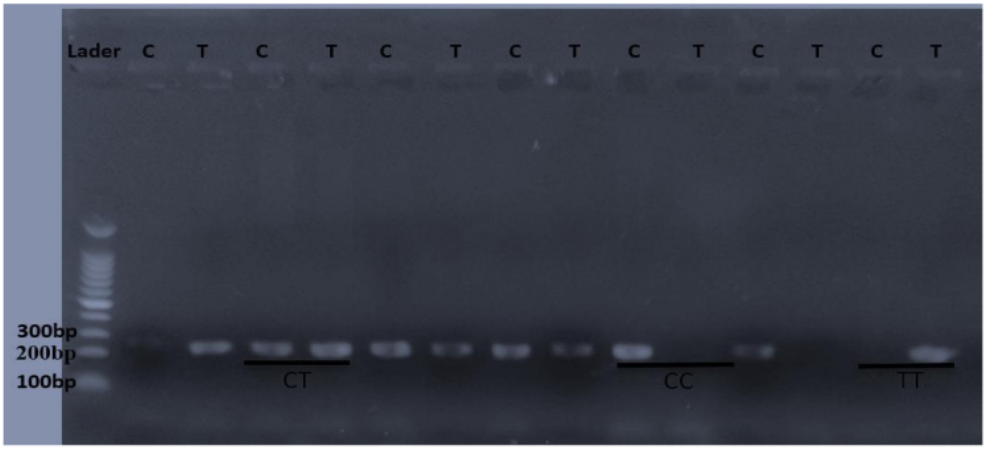

Figure (3) Electrophoresis of the mutant gene IL-4 -590 (C>T) showing the T and C alleles in patients with type 1 diabetes T2D.

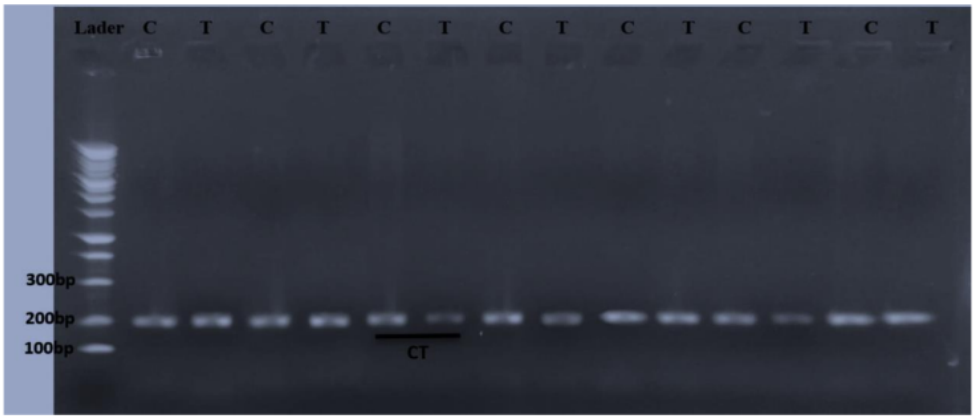

Figure (4) Electrophoresis of the mutant gene IL-4 -590 (C>T) showing the T and C alleles in the standard sample.

The results of the frequency distribution of the alleles of the mutant gene IL-4 -590, namely the t and c alleles, were different when using the Hardy-Weinberg equilibrium. The t allele recorded a rate of 51.67% in T2D patients. In contrast, its rate in the control sample was 50%, which is the highest. The critical ratio OR was 1.08 with a confidence interval (CI) of 95% ranging from 0.62

to 1.88, which is associated with the risk of developing the disease, which could constitute a potential risk factor. Its rate as a protective allele from the disease (PF) was 0.08 compared to the c allele. The latter rate in T2D patients was 48.33%, which is the highest compared to the control sample, which recorded a rate of 50%. The critical ratio OR is 0.92 with a CI confidence interval below the 95% significance level ranging from 0.53-1.60. Its proportion as a disease-causing allele EF reached 7.96%, as in Table (9). The results show no significant difference in the frequency distribution between the T2D patients sample and the standard sample in both alleles t and c using the Fishers test.

Table (9) Frequencies of the t and c alleles of the mutant gene IL-4 -590 (C>T) in T2D diabetic patients and the standard sample.

The gene	Allele	T1D patients (%)	Standard sample (%)	OR* (95%CI†)	P value
IL-4-590 (C>T)	T	51.67	50	1.08(0.62-1.88)	**0.77**
	P.F‡	-0.08%			
	C	48.33	50	0.92(0.53-1.60)	
	E.F§	7.69%			

OR* = Odd ratio (Critical ratio), † CI = Confidence Intervals (Confidence interval), P.F‡ = Preventive Faction (Protective Faction), § E.F = Ethiological Faction

Genetic analysis of the ARMS-PCR results of the mutant gene IL-4 -590 (C>T) using the Hardy-Weinberg equilibrium showed the presence of three genotypes in T2D diabetic patients and the standard sample, namely TT, CT, and CC. The percentage of the TT genotype in T2D diabetic patients was 6.67%. In comparison, its percentage in the standard sample was 0%, with a significant difference between them of 0.05 at a significance level of P<0.05 using the Fishers test. On the other hand, the value of the critical ratio OR was equal to 16.12, and the confidence interval CI value ranged from 0.90 to 28.23 at a significance level of 95%. This is considered a potential risk factor for the disease. As for its value as a protective genotype for T2D diabetes, its percentage was 15.12-. The results also showed high percentages of the CT genotype. Its percentage in T2D patients was 90%, and in the standard sample, it was 100%. By conducting statistical analysis, it

was found that there was a significant difference in the frequency of the presence of the CT genotype in T2D patients and the standard sample using the Fishers test at a probability level of 0.05 P<0.030, noting that the critical ratio OR was 0.04. The confidence interval CI ranged from 0.00 to 0.74 under a significance level of 95%, and its percentage as a protective pattern was 0.95%. This shows the potential protective role of this CC genotype as a potential influential factor in the incidence of T2D. Its percentage as a causative factor for the disease was 0.87%. As for its percentage in T2D patients, it is 3.33. In contrast, its percentage in the standard sample is 0%. Statistical analysis using the Fishers test showed no significant differences in the frequency of genotype CC in T2D patients compared to the standard sample at the probability level P<0.05, which is 0.19. The critical ratio RO for this pattern reached 7.21, and the confidence interval CI ranged from 0.36 to 14.53 at the probability level P<0.05, Table (10).

Table (10) Frequencies of genotypes of the mutant gene IL-4 -590 (C>T) in T2D diabetic patients and the standard sample.

The gene	Gene type	T1D patient%	Standard sample %	OR* 95% CI†	P value
IL-4-590 (C>T)	TT	6.67	0	16.12(0.90-28.23)	**0.41**
	PF‡	-15.12%			
	CT	90	100	0.04(0.00-0.74)	**0.61**
	PF‡	0.95%			
	CC	3.33	0	7.21(0.36-14.53)	**0.96**
	EF§	0.87%			

OR* = Odd ratio (Critical ratio), † CI = Confidence Intervals (Confidence interval), P.F‡ = Preventive Faction (Protective Faction), § EF = Ethiological Faction.

DISCUSSION

One of the most important causes of the development of type 1 diabetes mellitus (T1D) and type 2 diabetes mellitus (T2D) is immune and genetic interactions. In recent years, research has focused more on the immune and genetic role of interleukins, including anti-inflammatory interleukins. Interleukin 4 (IL-4) is an essential part of regulating the immune response. It is an anti-

inflammatory cytokine that participates in regulating the immune system at different levels. It has the ability to modify the differentiation, proliferation and apoptosis of groups of blood-forming and non-hematopoietic cells, in addition to T and B lymphocytes [9]. IL-4 is produced mainly by activated T cells, in addition to mast cells, basophils and eosinophils. Its molecular weight ranges between 12 and 20 kDa [10].

The results of this study showed a decrease in IL-4 concentrations in T1D patients (significant with the control sample). It also showed a decrease in its concentration in T2D patients (but not significant with the control sample). The results of the research also showed that T1D patients have CC and CT genotypes associated with the risk of developing the disease. As for the TT genotype, it may be a potential protective factor against developing the disease. The TT and CC genotypes that appeared in T2D patients were considered a potential risk factor and are associated with the risk of developing T2D. The CT genotype has a protective role against developing T2D. In general, the common genetic combination of genotypes that are associated with the risk of developing the disease is the CC genotype in both types of diabetes patients.

The release of anti-inflammatory cytokines, including interleukin-4 (IL-4), plays a role in protecting the pancreas and pancreatic beta cells (β cells) and preventing destructive islet inflammation. It contributes to stimulating various transcription factors and signal transduction pathways within beta cells (β cells) in addition to its ability to confront interleukin (IL-1β) (pro-inflammatory) and reduce nitrogen oxide (NO). In a study conducted on insulin-producing RINm5F cells cultured on RPMI1640 medium under appropriate laboratory conditions, and when testing the effect of IL-4 alone on these cells previously exposed to pro-inflammatory cytokines, the ability of IL-4 to prevent the loss of viability of insulin-producing cells was observed [11]. In the same context, the loss of beta cells is associated with increased secretion of pro-inflammatory cytokines released by type 1 helper T cells (Th1) in the islet environment. In contrast, this is accompanied by a decrease in anti-inflammatory cytokines, including IL-4, which is secreted by Th2 helper cells. Experiments on animal models have proven the immune reactions that occur between Th1 cells and Th2 cells, especially those that address the role of IL-4 [12].

The results of our current study on the decrease of IL-4 concentration in T1D diabetic patients are consistent with studies conducted on T1D diabetic patients and NOD mice. A decrease in IL-4 concentration was also observed. It was also reported that the overexpression of IL-4 or its

systemic administration prevents islet inflammation and reduces the incidence of T1D diabetes [1]. Another study agreed with the results of our study in the decrease of IL-4 concentration compared to the control sample. However, these results had non-significant differences in the level of IL-4 concentration [13]. The results of our current study are consistent with another study that showed the emergence of three genotypes of interleukin 4 also in T1D diabetic patients, namely TT, CT, and CC. The TT genotype was associated as a protective genotype against the risk of developing T1D. However, it differed from our study regarding the CT genotype, which was considered to have a major role as a protective aspect against developing the disease. It showed that the CC genotype is associated with the risk of developing T1D, and this is consistent with the results of our study [14].

Diabetes mellitus (T2D) is one of the most common diseases in the world. Research has revealed an increase in acute immune response and pro-inflammatory cytokines since 1997. Since then, research has considered this disease to be a chronic inflammatory disease. Several predisposing factors, including metabolic and genetic factors, overnutrition, aging, or changes in the gene expression of cytokines, trigger it. Pro-inflammatory cytokines cause damage to pancreatic islet cells. This leads to an imbalance of pro- and anti-inflammatory cytokines. IL-4 plays an important role in the pathophysiology of T2D diabetes [15]. The results of our current study showed a non-significant decrease in IL-4 concentration compared to the control sample. This result is consistent with a study conducted in Iraq in Wasit Governorate. The differences between IL-4 concentration in male T2D diabetic patients and the control sample were not significant [16]. In the genetic aspect of our study, there were three genotypes of the mutant gene for interleukin 4 IL-4-590 (C>T). The CT genotype was shown to have a potential protective role against T2D. The TT and CC genotypes were considered as a potential risk factor for T2D. The TT genotype had the highest incidence among the genotypes. Our results were consistent with a study conducted in northern Indonesia, which showed that the TT genotype had the highest incidence among T2D patients [17]. Other researchers found that the heterozygous genotype of the IL-4 gene may be a risk factor for T2D. On the other hand, they considered that the homozygous genotypes may be protective factors against T2D [18, 19]. Authors concluded that in patients with DM, the NLR, PLR, and older age were found as independent factors predicting CAD [20].

CONCLUSIONS

The results of our current study showed a decrease in the concentration of IL-4 in both T1D and T2D diabetic patients when compared with its concentrations in control samples. This may be attributed to the genotypes of the mutant gene IL-4-590 (C>T). The genotypes CC and CT were considered potential risk factors for T1D diabetes. The TT genotype was considered a potential protective factor for T1D diabetes. The TT and CC genotypes are risk factors for T2D diabetes. The CT genotype has a potential protective role for T2D diabetes.

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