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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 that leads to the destruction of pancreatic beta cells by immune cells. Type 2 diabetes (T2D) is a heterogeneous metabolic disorder characterized by hyperglycemia due to insulin deficiency or impaired secretion. It is also considered a chronic inflammatory disease. Interleukin 4 (IL-4) plays an anti-inflammatory role and is secreted by various immune cells. Genetic variations in the IL-4 gene, particularly at position 590 C>T, have been proposed as potential risk factors for diabetes.

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

Methods. Blood samples were collected from 30 male patients with type 2 diabetes and 24 non-diabetic controls. IL-4 concentrations were measured using the sandwich ELISA technique. Gene polymorphisms were detected using the amplification-refractory mutation system (ARMS) analysis.

Results. IL-4 concentrations were significantly decreased in T1D patients compared to controls (P = 0.022). In T2D patients, IL-4 levels were lower but not significantly different from controls (P = 0.52). Significant differences in IL-4 levels were observed between T1D and T2D groups. The TT and CT genotypes appeared to have a protective effect against T1D and T2D, whereas the CC and CT genotypes were considered potential risk factors.

Conclusion. Genetic variation in the IL-4 (590 C>T) region may contribute to reduced IL-4 levels and influence susceptibility to T1D and T2D.

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 (Th1) cells and others [1]. Type 2 diabetes (T2D) is a heterogeneous metabolic disorder caused by hyperglycemia resulting from insulin deficiency, secretion defects, or both [2]. It is also considered a chronic inflammatory condition. Triggers such as genetic predisposition, overnutrition, or aging can elevate cytokine expression levels [3].

Interleukin 4 (IL-4) is one such cytokine that has garnered attention for its anti-inflammatory properties. It is produced by immune cells such as T helper cells, mast cells, and others [4]. Since immune dysfunction is central to the pathogenesis of diabetes, current treatments are largely based on exogenous insulin replacement. This highlights the potential of immunotherapy in improving outcomes and disease management.

Genetic studies have emphasized the role of cytokines in T1D pathogenesis, where some cytokines drive inflammation while others modulate it by affecting beta cell destruction [3]. Genetic variation in the IL-4 gene region (590 C>T) has been identified as a potential risk factor in autoimmune diseases, particularly single nucleotide polymorphisms (SNPs) [5]. The gene encoding IL-4 is located on the long arm of chromosome 5 at locus 5q31. It spans approximately 0.9 kb and includes 4 exons [6]. IL-4 is synthesized as a 129-amino acid polypeptide and plays key roles in gene regulation, cell proliferation, apoptosis, and differentiation of various hematopoietic cells. Multiple studies suggest that polymorphisms at the 590 position may influence IL-4 production [7].

This study aimed to evaluate IL-4 concentrations and assess polymorphism at the IL-4 590 C>T gene re-

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gion, as well as their association with type 1 and type 2 diabetes.

TABLE 1. Primers used to detect IL-4 gene polymorphisms

	Primer name	Nucleotide sequence
1	T allele	5`GAATTT¬GTTAGTAATGCAGTCCTCC-3`
2	C allele	5`ACACTA¬AACTTGGGAGAACATTGTC-3`
3	Reverse	5`GAATTT¬GTTAGTAATGCAGTCCTCC-3`

TABLE 2. IL-4 concentration in the serum of T1D patients and control samples

Cytokinesis	mean ± standard error ng/L 30 samples for 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

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	

TABLE 4. IL-4 concentration in the sera of T2D samples and standard samples

Cytokinesis	mean ± standard error ng/L 30 samples for 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	9.45 ± 138.81	0.00	319.4	159.09 ± 9.06	106.31	222.77	0.52

MATERIALS AND METHODS

Blood samples were collected from 30 individuals with T1D, aged 3 to 30 years, and 30 individuals with T2D, aged 30 to 68 years, along with 24 non-diabetic controls. Samples were randomly collected from Al-Shifa Teaching Hospital, Al-Mawain Teaching Hospital, and Al-Faihaa Teaching Hospital in Basrah, Iraq, between January 25 and late July 2022.

From each participant, 5 ml of blood was drawn. 3 ml were collected in plain tubes for serum separation and subsequent IL-4 analysis using the sandwich ELISA technique (BT LAB, China), following manufacturer instructions.

For the molecular study, 2 ml of blood were collected in EDTA tubes for DNA extraction using the FAVORGEN BIOTECH CORP kit (Taiwan). IL-4 gene polymorphism at position -590 (C>T) was assessed using the ARMS-PCR method with Go Taq® Green Master Mix (Promega, USA). Three primers (Table 1) were used as described in [8].

The total PCR volume was 25 µL, including 12.5 µL of master mix, 2 µL of each primer, 5 µL of DNA template, and nuclease-free water. Thermal cycling included initial denaturation at 96°C for 1 minute, followed by 10 cycles of 95°C (15 s), 65°C (50 s), 72°C (40 s), then 20 cycles of 95°C (50 s), 59°C (50 s), and 72°C (50 s), with a final extension at 72°C for 7 minutes.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. For normally distributed data (e.g., IL-4 concentrations in T1D vs controls), the t-test was used, while for non-normally distributed data (e.g., T2D vs controls), the Mann–Whitney U test was applied. A p-value of <0.05 was considered statistically significant. Genotype and allele distributions were assessed using Hardy–Weinberg equilibrium, and Fisher's exact test was used to test significance. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using Compare version 3.04.

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	

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% CI		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	

RESULTS

IL-4 concentration in T1D patients

Table 2 shows a decrease in IL-4 concentration in the blood serum of T1D diabetic patients compared to the control group. Statistical analysis revealed a significant difference in IL-4 levels between T1D patients and controls (P < 0.05).

Age-related analysis (Table 3) showed that IL-4 levels were lower in patients aged ≤15 years com-

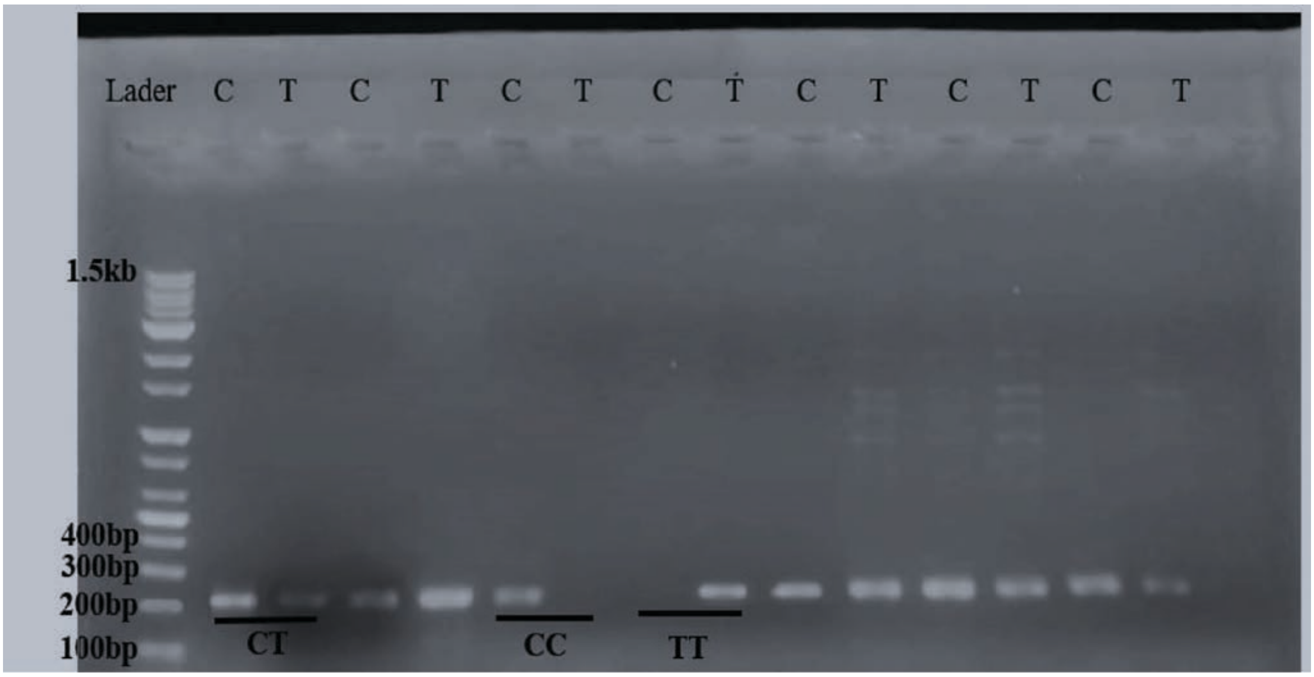

FIGURE 1. Electrophoresis of the IL-4 -590 (C>T) mutant gene showing the T and C alleles in patients with type 1 diabetes (T1D)

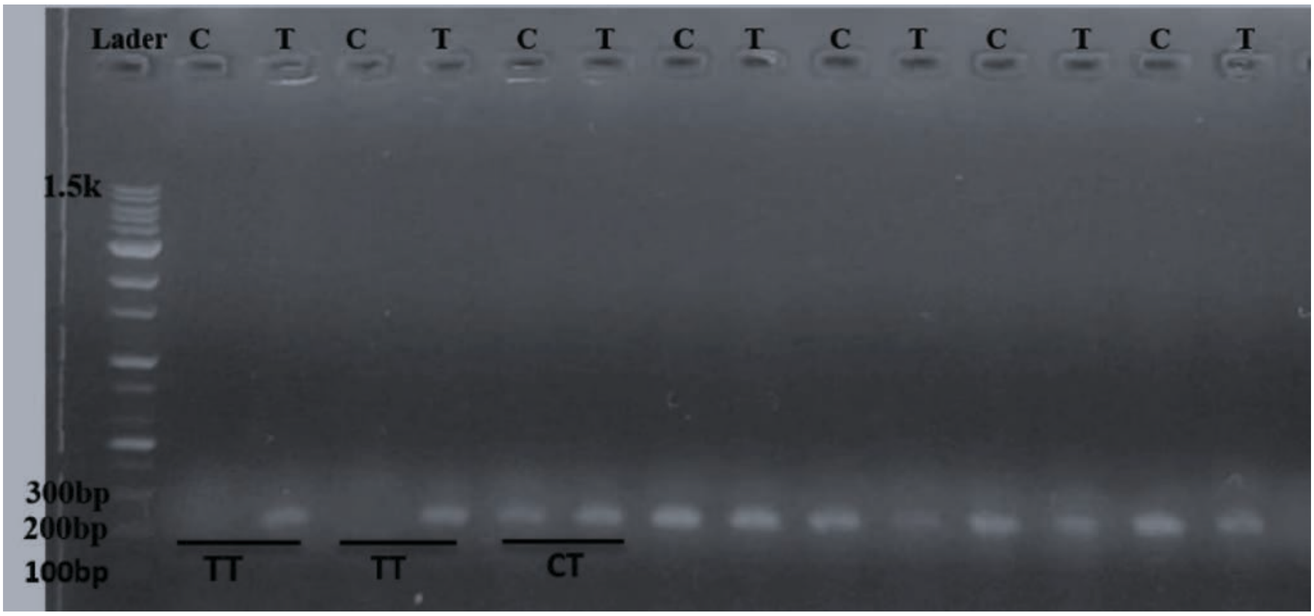

FIGURE 2. Electrophoresis of the IL-4 -590 (C>T) mutant gene showing the T and C alleles in control samples

pared to those >15 years. However, this difference was not statistically significant (P = 0.345).

IL-4 concentration in T2D patients

IL-4 levels were also reduced in T2D patients compared to controls, but the difference was not statistically significant (P = 0.52, Table 4).

Analysis by age group (Table 5) showed no significant difference in IL-4 concentration between T2D patients aged ≤45 and >45 years (P = 0.626).

Comparison between T1D and T2D patients

When comparing IL-4 concentrations between T1D and T2D patients, a statistically significant difference was observed (P = 0.025, Table 6), with lower levels in the T1D group.

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

T1D diabetic patients vs. control sample

The IL-4 gene at position -590 (C>T) was amplified using ARMS-PCR. The resulting PCR products were analyzed by electrophoresis. Two alleles, T and C, were detected in both T1D patients (30 samples) and control subjects (10 samples). Three genotypes were observed in T1D patients: CT, CC, and TT, while only CT and TT were found in controls (Figures 1 and 2).

According to Hardy–Weinberg equilibrium analysis, the T allele was more frequent in controls (60%) than in T1D patients (53%). The C allele was slightly more prevalent in T1D patients (47%) than in controls (40%) (Table 7). However, Fisher's exact test showed no significant difference in allele frequencies between the groups.

- The T allele had an odds ratio (OR) of 0.44 (95% confidence interval [CI]: 0.06–3.14), suggesting a 56% preventive fraction (PF).
- The C allele had an OR of 0.88 (CI: 0.63–1.23) with an etiological fraction (EF) of -0.1248, indicating a potential protective role rather than disease association.

For genotype frequencies, the TT genotype was found in 10% of T1D patients and 20% of controls. Fisher's test showed no significant difference (P = 0.41, OR = 0.44, CI: 0.06–3.14, PF = 56%).

The CT genotype was most common in both groups: 86.7% in T1D and 80% in controls. Fisher's test showed no significant difference (P = 0.61, OR = 1.62, CI: 0.24–10.57, PF = -0.625), suggesting it is not associated with increased risk.

The CC genotype was rare, present in 3.3% of T1D patients and absent in controls. Again, no significant difference was found ($P < 0.05$, OR = 1.06, CI: 0.04–28.29, EF = 6.35%, Table 8).

TABLE 7. Frequencies of the T and C alleles of the IL-4 -590 (C>T) mutant gene in T1D patients and control samples

The gene	Allele	T1D patients (%)	Control sample (%)	OR* (95%CI[†])	p-value
L-4-590 (C>T)	T	53%	60%	0.44 (95% CI: 0.06–3.14)	**0.41**
	P.F[‡]	(1 – 0.44) × 100 = 56%			
	C	47%	40%	0.88 (95% CI: 0.63–1.23)	
	EF[§]	- 0.12			

OR = odds ratio, [†]CI = confidence intervals, P.F[‡] = preventive fraction, [§]EF = etiological fraction*

TABLE 8. Frequencies of IL-4 -590 (C>T) genotypes in T1D patients and control samples

The gene	Gene type	T1D patients (%)	Control sample (%)	OR* (95%CI[†])	p-value
L-4-590 (C>T)	TT	10	20	0.44 (95% CI: 0.06-3.14)	**0.41**
	P.F[‡]	56%			
	CT	86.7	80	1.62 (95% CI: 0.24-10-57)	**0.61**
	P.F[‡]	-0.62			
	CC	3.3	0	1.06 (95% CI: 0.04-28.29)	**0.96**
	EF[§]	6.35%			

OR = odds ratio, [†]CI = confidence intervals, P.F[‡] = preventive fraction, [§]EF = etiological fraction*

T2D diabetic patients vs. control sample

Amplification of the IL-4 gene at the -590 (C>T) site was performed for 30 T2D patient samples and 10 control samples using ARMS-PCR. Electrophoresis revealed both T and C alleles. The genotypes CT, TT, and CC were identified in T2D patients, while only the CT genotype appeared in controls (Figures 3 and 4).

The T allele frequency was slightly higher in T2D patients (51.67%) than in controls (50%), with an OR of 1.08 (CI: 0.62–1.88) and PF = 0.08, suggesting a potential risk factor (Table 9).

The C allele was present at 48.33% in T2D patients and 50% in controls (OR = 0.92, CI: 0.53–1.60, EF = 7.96%). Fisher's test showed no significant differences in allele distribution between groups.

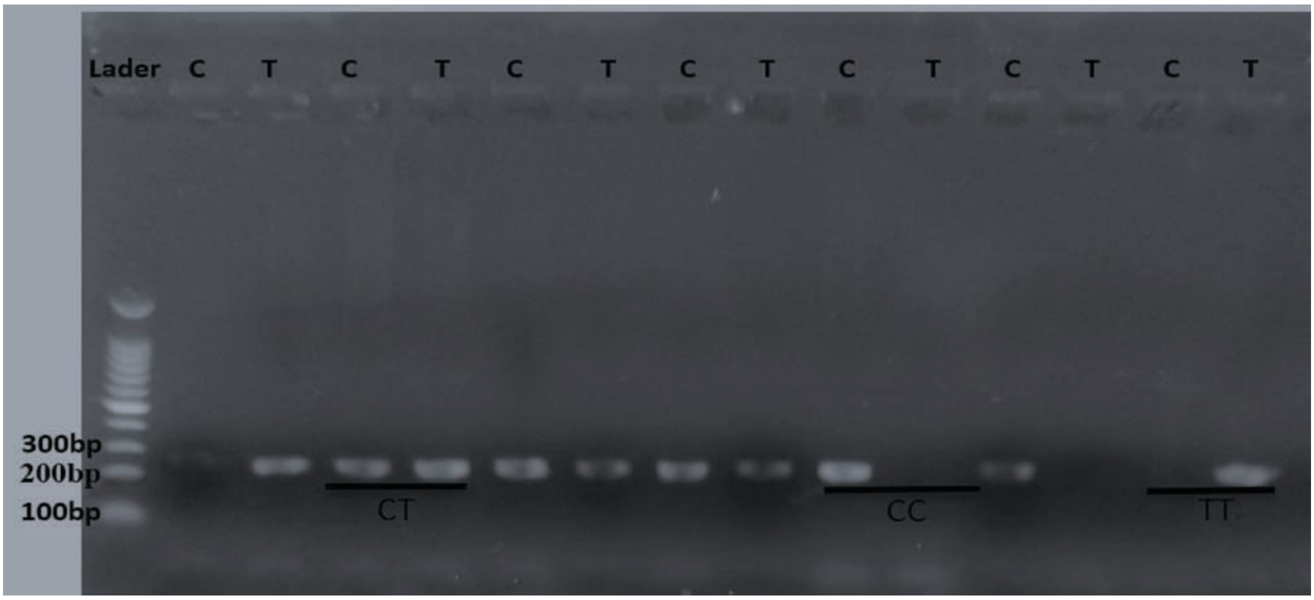

FIGURE 3. Electrophoresis of the IL-4 -590 (C>T) mutant gene showing the T and C alleles in patients with type 2 diabetes (T2D)

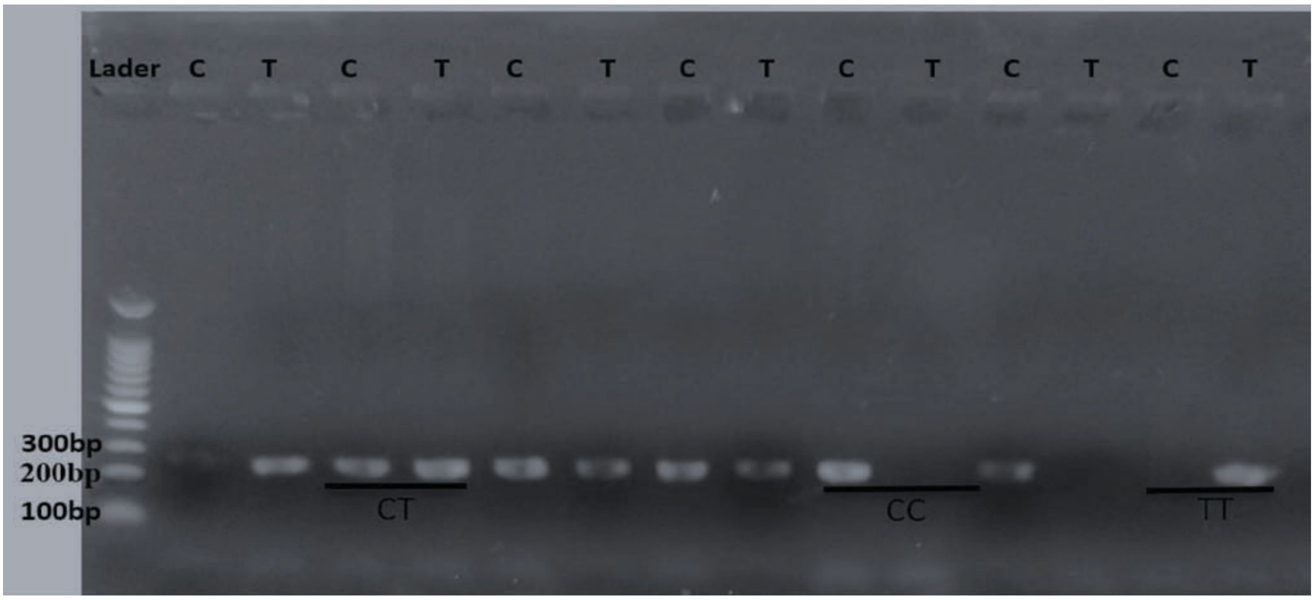

FIGURE 2. Electrophoresis of the IL-4 -590 (C>T) mutant gene showing the T and C alleles in control samples

Genotypic analysis showed:

- TT genotype occurred in 6.67% of T2D patients and 0% of controls. Fisher's test showed a significant difference (P = 0.05, OR = 16.12, CI: 0.90–28.23), indicating it may be a potential risk factor (PF = -15.12%).
- CT genotype was found in 90% of T2D patients and 100% of controls. Fisher's test showed a significant difference (P < 0.030, OR = 0.04, CI: 0.00–0.74, PF = 0.95%), suggesting a protective effect.
- CC genotype appeared in 3.33% of T2D patients and was absent in controls. This difference was not statistically significant (P = 0.19, OR = 7.21, CI: 0.36–14.53, EF = 0.87%, Table 10).

DISCUSSION

One of the most important contributors to the development of type 1 diabetes mellitus (T1D) and type 2 diabetes mellitus (T2D) is the interaction between immune and genetic factors. In recent years, research has increasingly focused on the immunological and genetic roles of interleukins, particularly those with anti-inflammatory properties. Interleukin-4 (IL-4) plays an essential role in regulating the immune response. It is an anti-inflammatory cytokine that modulates immune activity at various levels. It has the ability to influence differentiation, proliferation, and apoptosis in both blood-forming and non-hematopoietic cells, including T and B lymphocytes [9]. IL-4 is produced mainly by activated T cells, as well as mast cells, basophils, and eosinophils, and has a molecular weight ranging between 12 and 20 kDa [10].

TABLE 9. Frequencies of the T and C alleles of the IL-4 -590 (C>T) mutant gene in T1D patients and control samples

The gene	Allele	T2D patients (%)	Control sample (%)	OR* (95%CI†)	p-value
L-4-590 (C>T)	T	51.67	50	1.08 (95% CI: 0.62-1.88)	**0.77**
	P.F‡	-0.08%			
	C	48.33	50	0.92 (95% CI: 0.53-1.60)	
	EF§	7.69%			

OR = odds ratio (critical ratio), †CI = confidence intervals (confidence interval), P.F‡ = preventive fraction (protective fraction), §EF = etiological fraction*

The findings of this study revealed a significant decrease in IL-4 concentrations in T1D patients compared to controls. In T2D patients, IL-4 levels were also reduced, but the difference was not statistically significant. Genotypic analysis in T1D patients showed that the CC and CT genotypes were associated with an increased risk of developing the disease, whereas the TT genotype may act as a potential protective factor. In T2D patients, both TT and CC genotypes were identified as potential risk factors, while the CT genotype showed a protective association. Overall, the CC genotype was commonly associated with an increased risk in both types of diabetes.

The release of anti-inflammatory cytokines, including IL-4, plays a protective role in preserving pancreatic beta cells and reducing destructive inflammation within the islets. IL-4 activates transcription factors and signaling pathways in beta cells and can counteract the effects of pro-inflammatory cytokines such as IL-1β, as well as reduce levels of nitric oxide (NO). In a study involving insulin-producing RINm5F cells cultured in RPMI 1640 medium, IL-4 was shown to protect these cells from cytokine-induced loss of viability [11].

Similarly, beta cell loss in T1D is associated with an increased secretion of pro-inflammatory cytokines by Th1 cells, and a corresponding decrease in anti-inflammatory cytokines like IL-4, which is secreted by Th2 cells. Animal models have confirmed the immunological interplay between Th1 and Th2 cells, particularly highlighting the regulatory role of IL-4 [12].

Our current findings on the reduction of IL-4 in T1D patients are consistent with previous studies conducted in both T1D patients and NOD mice, which also reported decreased IL-4 levels. Additional studies have shown that overexpression or systemic administration of IL-4 can prevent islet inflammation and reduce T1D incidence [1]. Another study aligned with our findings by reporting decreased IL-4 levels in T1D patients, though the differences were not statistically significant [13]. Furthermore, one study also observed the presence of the TT, CT, and CC genotypes in T1D patients. The TT genotype was linked to a protective effect, while the CC genotype was associated with disease risk, consistent with our results. However, their interpretation of the CT genotype differed; they considered it protective, unlike our findings [14].

T2D is among the most prevalent metabolic disorders worldwide. Since 1997, research has recognized T2D as a chronic inflammatory disease, characterized by an increased immune response and elevated pro-inflammatory cytokines. Various factors – including genetic and metabolic predisposition, overnutrition, aging, and altered cytokine gene expression – can trigger inflammation that damages pancreatic islet cells, leading to an imbalance between pro- and anti-inflammatory mediators. IL-4 plays a significant role in the pathophysiology of T2D by contributing to this regulatory balance [15].

Our study showed a non-significant decrease in IL-4 concentration in T2D patients compared to controls. This is consistent with a study conducted in Wasit Governorate, Iraq, which also found no significant difference in IL-4 levels between male T2D patients and controls [16]. Genotyping analysis revealed the presence of TT, CT, and CC genotypes in T2D patients. The CT genotype was associated with a protective role, while TT and CC genotypes were considered potential risk factors. The TT genotype had the highest incidence among T2D patients in our study, a finding that agrees with a study from northern Indonesia [17].

However, other researchers reported contrasting results, suggesting that the heterozygous CT genotype may represent a risk factor, while the homozygous TT or CC genotypes might offer protection against T2D [18,19]. Another study concluded that in diabetic patients, NLR (neutrophil-to-lymphocyte ratio), PLR (platelet-to-lymphocyte ratio), and older age were independent predictors of coronary artery disease (CAD) [20].

Limitations

This study has certain limitations that should be considered when interpreting the results. First, the sample size was relatively small, particularly in the subgroup analyses of IL-4 genotypes, which may have reduced the statistical power to detect weaker associations. In addition, the number of control subjects included in the genetic analysis was limited, which could affect the generalizability of the observed genotype distributions. Second, the study population was restricted to a single geographic region in southern Iraq, which may not reflect broader genetic or environmental diversity. Future studies with larger, more diverse cohorts and functional assays are recommended to validate and expand upon these findings.

CONCLUSIONS

The results of our study showed a decrease in IL-4 concentrations in both T1D and T2D patients compared to control samples. This reduction may be associated with polymorphisms in the IL-4 -590 (C>T) gene. The CC and CT genotypes were identified as potential risk factors for T1D, while the TT genotype appeared to have a protective effect. In T2D patients, the TT and CC genotypes were considered risk factors, whereas the CT genotype showed a potential protective role.

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