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Original Research

Serum levels of TNF-α and IFN-γ gene polymorphism in type 2 diabetes mellitus in kurdish patients

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Abstract: Type 2 diabetes mellitus (T2DM) is a metabolic disease and cytokines show a vital role in the T2DM progress. The goal of this research was to assess serum levels of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) gene polymorphism in T2DM in Kurdish patients. Levels of serum IFN-γ and TNF-α were assessed through enzyme-linked immune sorbent assay in individuals with T2DM and the control group. DNA was extracted and the amplification refractory mutational system method was utilized for genotyping the IFN-γ (+874) A/T and TNF-α (-308) G/A. The Hardy–Weinberg equilibrium was evaluated with the χ2-test. The IFN- γ serum levels were significantly different between patients with T2DM and control individuals (P<0.05). But the C-reactive protein (CRP) and TNF-α serum levels were not significantly different between them (P>0.05). The serum level of IFN- γ (+874) AT genotype and TNF-α (- 308) GG genotypes were significantly higher in the T2DM group comparing with healthy people (P<0.05). A significant relation between T2DM and IFN-γ (+874) gene polymorphism's TT and AT genotypes was observed. Also, it was not a significant relation between TNF-α (−308) gene polymorphism's GG and GA genotypes and T2DM. But the statistically significant difference was found in the genotype AA frequency. Genetic polymorphisms of IFN-γ (+874) and TNF-α (−308) are contributed to the genetic susceptibility for T2DM development in the Kurdish population. Early screening of these two genetic polymorphisms may assist in the early control and management of T2DM.

Key words: TNF-α; IFN-γ; T2DM; Kurdish people; Polymorphism.

Introduction

Defects in insulin action can result in impaired glucose metabolism that causes chronic metabolic disease, type 2 diabetes mellitus (T2DM) (1). It was approximated that there are about 463 million individuals with diabetes mellitus globally in 2019 and the prevalence of diabetes is expanding around the world (2). Different environmental factors like obesity or physical inactivity and genetic factors are involved in the impaired homeostasis of glucose in T2DM (3). Approximately 80% of individuals with T2DM are overweight (4).

It has been recognized that cytokines have a crucial effect on T2DM. They regulate immune response and different factors affect the cytokine expression via immune cells. Some of these factors are hormonal conditions, inflammation, infection and gene polymorphisms (5). Cytokines including IL-6, IFN-γ and TNF-α have been stated as effective factors for diabetes development. In addition, the effect of genetic polymorphism on pro-inflammatory and anti-inflammatory specific cytokine genes is a regular risk factor for diabetes development (6, 7). Enhancing levels of these pro-inflammatory cytokines result in hepatic secretion and production of proteins like amyloid-A, plasminogen activator inhibitor, and C-reactive protein (CRP). At the early stages of T2DM, these proteins appear, then their concentrations enhance with developing the disease (8).

A cytokine is Tumor Necrosis Factor-alpha (TNF-α) is located into the extremely polymorphic area on chromosome 6p21.3. TNF- α affects the regulation of apoptosis, differentiation and cell proliferation (6, 9). The TNF-α primary source is macrophages. Various cells including tumor cells, endothelial cells, B and T cells, smooth muscle cells and osteoblasts can secret TNF-α (6, 10). There are various studies state that inflammatory conditions such as T2DM are related to single nucleotide polymorphism at -308 G/A (5, 11). In addition, it was demonstrated that TNF neutralization enhances insulin sensitivity (12). Also, it was shown in different studies that TNF-α prevents insulin receptors from signaling (6, 13). In addition, increasing the gene transcription can occur as a result of a G nucleotide replacement by A in the TNFα gene promoter (−308 G/A). As a result, TNF-α (−308) A/A show decreased levels of HDL and enhanced levels of fat comparing with (−308) G/A (14). The production of TNF-α cytokine increase as a result of high gene transcription which can cause T2DM occurrence (6, 15). Therefore, investigating the TNF-a role in influencing T2DM is very important and polymorphic genotype can be related to an enhanced T2DM frequency.

IFN- γ is a cytokine that supports target cell cytolysis by the immune system and it is enhanced in T2DM (5, 16). IFN- γ through up-regulating various pro-inflammatory mediators like IL-6 and TNF-α can direct the inflammatory response (17). It was reported that a T nucleotide replacement by A at position +874 is associated with enhanced expression of IFN- γ (17, 18). Also, it was observed that there is a relation between T2DM and

the polymorphisms of the IFN- γ gene +874 (A/T) (19).

Therefore, this research aimed to evaluate serum levels of IFN-γ at (+874) A/T and TNF-α at (-308) G/A gene polymorphism in T2DM in Kurdish patients.

Materials and Methods

Patients and control groups

The present study included 180 individuals. All were from Erbil province in northern Iraq. The patients attended to Layla Qassim diabetic center, Erbil city, Kurdistan Region, Iraq. The study population was classified into two treatments. First treatment includes 90 individuals of healthy volunteers (median = 47.96 years; min-max = 32-61 years; 54 women and 36 men). The control treatment was chosen from patients without T2DM or other metabolic diseases. Another treatment includes 90 patients with T2DM (median = 48.58 years; min-max = 30-65 years; 60 women and 30 men). Data of these individuals were collected from November 2020 to April 2021 from Diabetic Center in Erbil province. A questionnaire for recording different information like sex, age, social status, family history and duration of disease was designed and individuals were visited at the center. All of the study participants signed a consent form. Also, approval was achieved from the ethics committee of the Department of Biology, College of Education, Salahuddin University-Erbil, Kurdistan Region, Iraq.

Blood sample collection

Various diagnostic tests and clinical evaluations such as glycated hemoglobin, blood pressure, serum insulin, body mass index, and fasting blood sugar were performed to carry out clinical profiling of the patients. After 12-14 hours of night fasting, 7 ml of venous blood was collected from the antecubital vein of each person for biochemical tests. The supernatant was used for the measurement of glycated hemoglobin (HbA1c) by Tinaquant kit, serum insulin by Elecsys kit and estimation of fasting blood sugar by Glucose HK kit. Serum levels of IFN-γ and TNF-α were assessed using ELISA. The Cloud Clone Corp kit was applied base on the manufacturer's protocol. For measuring insulin resistance, the formula of homeostasis model assessment of insulin resistance (HOMA-IR) was applied.

Cytokine genotype

To achieve genomic DNA for T2DM patients and healthy individuals, the QLAmp DNA mini kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions for peripheral blood mononuclear cells. For the IFN-γ genotype at (+874 A/T) (rs2430561) and TNF-α genotype at (- 308 G/A) (rs1800629), the amplification refractory mutational system method (ARMS-PCR) was used. The examinations were done in a 20 μL reaction volume contains 1.5 Mm dNTPs, 40 ng genomic DNA, 25Mm MgCl2, 0.4 units of Tag polymerase (Fermentas, Maryland, USA) and 1 μL of 10 pmol each primer in 1 X Reaction Buffer. The sequence of primers was designed as follows: for generic primer of IFN-γ 5'-TCAACAAAGCTGATACTCCA-3'; IFN-γ (T) Allele Primer 5'-TTCTTACAACACAAAATCAAATCT-3'; IFN-γ (A) Allele Primer 5'-TTCTTACAACACAAAATCAAATCA 3' and for common primer of TNF-α 5'-TCCTCCCTGCTCCGATTCCG-3'; TNF-α (A) Allele Primer 5'-CAATAAGTTTTGAGGGGCATGA-3'; TNF-α (G) Allele Primer 5'-CAATAAGTTTTGAGGGGCATGG-3'. The reaction was performed in a thermal cycler with a cycling situation that includes: 95 C for 2 minutes, 35 cycles at 95 C for 45 seconds, 58 C for 40 seconds, 72 C for 1 min, and eventually a 7 minutes extension at 72 C. The amplicon size for IFN-γ 263bp was and for TNF-α was 104bp. The 2% agarose gel was used for analyzing the amplified products.

Statistical Analysis

Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) statistical package was used. Normally distributed variables were demonstrated as mean ± SD. The P value<0.05 was considered statistically significant. The ANOVA test was applied for between-group comparisons of categorical variables.

For gene polymorphism of TNF-α and IFN-γ, direct allele counting was used for counting the allele. Using the $\chi 2$-test, the Hardy–Weinberg equilibrium was evaluated. Frequencies of alleles and genotypes were compared between treatments through a $\chi 2$-test of independence with the z statistics and 2x2 contingency tables.

Results

For evaluating serum levels of TNF-α and IFN-γ gene polymorphism in T2DM in Kurdish patients, 180 participants (90 patients and 90 healthy people) were in-

Table 1. Characteristics of T2DM patients and control groups.

Characteristics	T2DM Mean± SD (N = 90)	Control Mean± SD (N = 90)	P - value
Age (years)	48.58±1.9	47.76±1.765	-
Gender (Male/Female)	60/30	54/36	-
Duration (years)	7.479±0.656	0	<0.0001
Family history n. (%)	26 (86.66)	8 (26.66)	-
BMI (kg/m^2)	29.263±1.024	20.608±0.377	<0.0001
Hypertension			
SBP	170.6±2.579	117.3±1.63	<0.0001
DBP	91.07±0.71	76±1.18	<0.0001
Fasting blood sugar (mg/dl)	206.417±1.77	88.52±2.919	<0.0001
HbA1c (%)	7.71±0.369	5.699±0.09	0.002
Insulin (pmole/1)	9.82±6.39	6.93±0.964	<0.0001
HOMA-IR	3.734±0.339	1.527±0.17	0.0003

BMI: body mass index, HbA1c: glycated hemoglobin.

cluded. Baseline specifications of patients with T2DM and control groups are demonstrated in Table 1.

Mean ± standard deviation (SD) of the age of individuals with T2DM and healthy people were 48.58±1.9 and 47.76±1.765 years, respectively. The gender distributions of Male/Female were 60/30 in T2DM patients and 54/36 in healthy individuals. Duration of disease in T2DM was 7.479±0.656 (Mean ± SD) years. Among the total 90 patients, 86.66% had a family history of diabetes, whereas 26.66% of healthy individuals had a family history of diabetes. It was not significantly different (P<0.05). BMI of people in both two groups was calculated and recorded. The BMI was 29.263±1.024 (Mean ± SD) for T2DM patients and 20.608±0.377 for healthy individuals. This difference was significantly different (P<0.05). Therefore, BMI has a significant effect on the occurrence of T2DM in this study

About hypertension, systolic blood pressure (SBP) was 170.6±2.579 in T2DM cases and 117.3±1.63 in controls. Diastolic blood pressure (DBP) in T2DM cases and controls was 91.07±0.71 and 76±1.18, respectively. The differences between the two groups were significant in both SBP and DBP (P<0.05). Therefore, hypertension is an effective factor in occurrences of T2DM.

The mean fast blood sugar (FBS) levels were much higher in T2DM cases (206.417±1.77 mg/dl) comparing with healthy controls (88.52±2.919 mg/dl) (P<0.05). Glycated hemoglobin (HbA1c) was 7.71% and 5.699% in T2DM patients and healthy individuals, respectively. The difference between the two groups in HbA1c was significant (P<0.05).

The insulin serum level in T2DM patients was significantly higher (9.82±6.39) compared to healthy controls (6.93±0.964) (P<0.05). The CRP serum levels were measured along with IFN- γ and TNF-α serum levels. The IFN- γ serum levels were significantly distinct between T2DM patients and control individuals (P<0.05). But the TNF-α and CRP serum levels were not significantly different between them (P>0.05) (Table 2).

In addition, the IFN- γ and TNF-α serum levels in patients with T2DM and healthy individuals were measured according to the genotypes. The serum level of IFN-γ (+874) AT genotype was significantly higher in the T2DM group comparing with healthy people (P<0.05). But serum levels of IFN- γ (+874) TT genotype were not significantly distinct between them (P<0.05) and IFN- γ (+874) AA genotype was not observed in the serum. The serum level of TNF-α (- 308) GG genotypes was significantly higher in T2DM patients compared to healthy controls. But serum levels of TNF-α (- 308) GA and AA genotypes were not significantly different between individuals with T2DM and the control group (Table 3).

Genotypic and allelic frequencies of gene polymorphism of TNF-α and IFN- γ were calculated through Hardy Weinberg equilibriums (Table 4). Among T2DM

Table 2. Serum level of IFN- γ, TNF-α and CRP in T2DM patients and control group.

Parameters	Patients	Control	P value
IFN-Y	74.75±10.35	26.27±2.32	<0.0001
TNF-α	9.19±1.88	4.74±0.36	0.97
CRP	0.43±0.06	0.17±0.05	0.98

Table 3. IFN- γ and TNF-α serum levels in T2DM patients and control individuals according to the genotypes.

Parameters	Patients	Control	P value
IFN- γ			
TT	36.88±4.0	53.5±2.0	0.9
AT	84.1±4.1	39.52±3.9	0.05
AA	-	-	-
TNF-α			
GG	247.5±35.3	33.21±3.12	0.0001
GA	33.65±10.66	45.43±5.0	0.9
AA	55.86±11.74	25.25±2.88	0.89

Table 4. Frequencies of alleles and genotypes of IFN-γ and TNF-α in T2DM individuals and healthy group.

IFN-γ		T2DM N (%)	Control N (%)	P	X^2
Genotypes	TT	72(80)	30(33.3)	0.0001	39.68
	AT	18(20)	52 (57.8)	0.0001	26.87
	AA	0	8 (8.9)	0.003	8.32
Alleles	T	162 (90)	112(62)	0.0002	38.08
	A	18 (10)	68 (38)	0.0002	38.08
TNF-α					
Genotypes	GG	54 (60)	55(61)	0.5	0.23
	AG	21 (23.4)	31 (34.5)	0.06	2.68
	AA	15 (16.6)	4 (4.5)	0.007	7.08
Alleles	G	129 (71.67)	95 (52.78)	0.001	13.62
	A	51 (28.33)	85 (47.22)	0.001	13.62

patients, for IFN- γ (+874) A/T genetic polymorphism, 72/90 (80%), 18/90 (20%) and 0/90 (0%) were TT, AT and AA genotypes, respectively. Likewise, from 90 healthy controls 30/90 (33.3%), 52/90 (57.8%) and 8/90 (8.9%) were TT, AT and AA genotypes, respectively. Significant associations between T2DM and IFN-γ (+874) gene polymorphism's TT and AT genotypes were observed (P<0.05). Contrary, there were not any significant differences in the frequency of genotype AA.

Similarly, the allelic frequency of +874*T and +874*A alleles in people with T2DM were 162 (90%) and 18 (10%) respectively. In addition, the allelic frequency of +874*T and +874*A were calculated to be 112 (62%) and 68 (38%) in control individuals, respectively. The differences between patients with T2DM and the healthy group in frequencies of both T and A alleles were significant (P<0.05).

For genetic polymorphism of TNF-α −308 G/A among T2DM patients, GG, AG and AA genotypes were 54/90 (60%), 21/90 (23.4%) and 15/90 (16.6%) respectively. Likewise, from 90 control individuals 55/90 (61%), 31/90 (34.5%) and 4/90 (4.5%) were GG, AG and AA genotypes, respectively. There was not a significant relation between TNF-α (−308) gene polymorphism's GG and GA genotypes and T2DM (P<0.05). But a significant difference was found in the frequency of genotype AA.

Also, the G and A allelic frequency of TNF-α (−308) in T2DM patients were 129 (71.67%) and 51 (25.33%) respectively. Also, the G and A allelic frequency of TNF-α (−308) were found to be 95 (52.78%) and 85 (47.22%) in healthy controls, respectively. The differences between patients with T2DM and healthy individuals in frequencies of both G and A alleles were significant (P<0.05).

Statistical evaluations of associations between IFN-γ +874 genotypes or alleles and Relative risk and Etiological or Preventive Fraction of T2DM patients were calculated and shown in Table 5. All of these associations were significant. All genotypes TT, TA and AA were observed to be significantly related to 8- and 0.18-fold enhanced risk for T2DM, respectively.

Statistical evaluations of associations between TNF-α-308 genotypes or alleles and Relative risk and Etiological or Preventive Fraction of T2DM patients were calculated and shown in Table 6. The association between AA genotypes and relative risk and Etiological or Preventive Fraction was significant.

Both alleles show significant association. Genotype AA was measured to be significantly associated with a 4.3-fold increased risk for T2DM.

Discussion

Cytokines regulate immune response and play key roles in T2DM. Immune cells express cytokines at the effect of different factors including hormonal condition, inflammation, infection, and gene polymorphisms of cytokines (20). It was demonstrated that pro-inflammatory cytokines, immune system activation, and inflammation have a crucial effect on the progress and pathogenesis of T2DM (21, 22). Nevertheless, the mechanisms of chronic inflammation in T2DM are not entirely realized.

In this research, we evaluate serum levels of TNF-α and IFN-γ gene polymorphism in T2DM in Kurdish patients. Also, we analyzed gene polymorphisms of cytokines of IFN-γ and TNF-α in T2DM patients. The gene regions which affect the expression of these genes were selected to compare between T2DM patients and control individuals.

No significant differences were observed between the healthy group and patients with T2DM in gender, age, and family history (P>0.05). Individuals with T2DM had significantly higher BMI, hypertension (SBP and DBP), fasting blood sugar, HbA1c, HOMA-IR and insulin.

The difference between the IFN- γ serum levels of T2DM patients and control individuals was significant (P<0.05). But the CRP and TNF-α serum levels were not significantly different between these two treatments (P>0.05).

The results of studies demonstrated that the synthesis of some anti-inflammatory and pro-inflammatory cytokines including TGF-β1, INF-γ, TNF-α, IL-10 and IL-6 have a crucial role in the pathogenesis and complications of T2DM (23, 24).

INF-γ that is involved in fibrosis development in inflamed tissues is an inflammatory cytokine. It was demonstrated that INF-γ is very important for host defense against various infections. The outcomes of the present

Table 5. Statistical evaluations of associations between IFN- γ +874 genotypes or alleles and T2DM patients.

IFN-γ $_{+874}$ Genotype or Allele	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
TT	8	0.7	Significance	4.08 to 15.69
TA	0.18	0.47	Significance	0.09 to 0.35
AA	0.05	0.08	Significance	2.5 to 14.8
T	5.46	0.73	Significance	3.09 to 9.67
A	0.18	0.3	Significance	0.10 to 0.32

Table 6. Statistical evaluations of associations between TNF-α-308 genotypes or alleles and T2DM patients.

TNF-α $_{-308}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
GG	0.95	0.02	Not significance	0.53 to 1.73
GA	0.58	0.14	Not significance	0.30 to 1.11
AA	4.3	0.12	Significance	1.38 to 13.43
G	2.26	0.4	Significance	1.46 to 3.50
A	2,45	0.16	Significance	1.44 to 4.17

research demonstrated that the IFN-γ level was higher in the serum of diabetic patients comparing with healthy controls. Various factors affect cytokine expression such as cytokine gene polymorphism, hormone conditions, inflammation and infection (25).

The outcomes of the present study are in the agreement with the study of Jagannathan- Bogdan *et al.* (2011), which demonstrated enhanced levels of INF-γ in patients with T2DM compared to healthy individuals (26). Also, the outcomes of a study demonstrated a significant enhancement of IFN-γ and an enhancing trend of CRP in diabetic individuals with no sex difference (27). The result of their study suggested that there can be a relation between the pro-inflammatory cytokines and the cause of T2DM (27).

No significant difference was observed between TNF-α and CRP serum levels in T2DM patients and controls. ($P > 0.05$). TNF-α is a cytokine that has a vital role in the regulation of apoptosis, differentiation and cell proliferation. Insulin signal pathways can be disrupted by TNF-α which causes B cell destruction. The increasing TNF-α plays a vital role in the progress of T2DM (28-30).

The results of some studies showed that the TNF-*α* serum level in T2DM patients was significantly higher than in healthy individuals (31, 32). There was a high positive correlation between serum levels of TNF-α and HbA1c, which was positively related to insulin resistance. They suggested that TNF-α has a significant effect on the pathogenesis of T2DM through mechanisms related to the environmental function of leptin-independent insulin. A meta-analysis of nineteen researches demonstrated that enhanced levels of inflammatory cytokines like TNF-α were highly related to the enhanced risk to the incidence of T2DM (33).

The serum level of IFN- γ AT genotype was significantly higher in T2DM patients comparing with healthy individuals ($P<0.05$). But serum levels of IFN- γ TT genotype were not significantly different between them ($P<0.05$) and IFN- γ AA genotype was not observed in the serum.

In a study that investigated the association of IFN- γ +874 T/A gene polymorphism with T2DM in rheumatoid arthritis (RA) patients, the IFN- γ level was significantly higher in these patients with and without T2DM compared to controls, also they indicated that IFN- γ T/A genotyping represented a significant enhance in the frequency of AA genotype and a significant reduction in TT genotype patients with T2DM compared to healthy people (17).

In this study, a significant relation between IFN-γ (+874) gene polymorphism's TT and AT genotypes and T2DM was observed. But, there were not any statistically significant differences in the frequency of genotype AA. Also, no significant relation was observed between TNF-α (−308) gene polymorphism's GG and GA genotypes and T2DM. But a significant difference was found in the genotype AA frequency.

The results of this study regarding gene polymorphism of IFN-γ+874 (A/T) demonstrated that the TT genotype was lower and the AT frequency was higher in Kurdish diabetic patients compared to healthy individuals. These outcomes are in contrast to the results reported by Pravica et al. (2000) and Tsiavou et al (2005) who reported enhanced levels of INF-γ +874 AA genotype in Greece diabetic patients compared to the healthy group (18, 19). This may draw attention to the influence of high INF- γ on the immune disturbance development that predisposes to T2DM.

In a study on the correlation between IL-4 and IFN-γ polymorphisms in T2DM, it was concluded that IFN-γ polymorphism is related to diabetes, but a significant relationship was not found between IL-4 polymorphism and diabetes (34).

It was found that IFN-γ is contributed to the pathogenesis of diabetes. The outcomes demonstrated that IFN-γ has a crucial influence on the progression and development of diabetes because it destroys beta cells of the islets of Langerhans and causes insulin resistance which leads to diabetes development (19). It was reported that IFN-γ +874 T / A gene polymorphism affects the expression of its gene. The AA genotype is related to low levels of IFN-γ comparing with the TT genotype, but the TA genotype showed moderate levels (18).

The TNF-α (- 308) GG genotype serum level was significantly higher in people with T2DM comparing with healthy individuals. But serum levels of TNF-α (-308) AA and GA genotypes were not significantly different between T2DM patients and the control group. Also, it was not a significant relation between TNF-α (−308) gene polymorphism's GG and GA genotypes and T2DM. But the statistically significant difference was observed in the genotype AA frequency.

Various researches have demonstrated the effect of different polymorphisms on proinflammatory cytokines genes as a risk factor for the progression of obesity, metabolic syndrome, and diabetes (35). TNF-α has been studied as one of these cytokines because diabetics increase circulating levels of TNF-α. TNF-α also causes insulin resistance, which is related to the pathogenesis of T2DM (36).

In our study, when comparing the frequencies of polymorphic genotypes, it can be seen that (AA) genotype is higher in T2DM patients. Therefore, this point mutation is considered a potential risk factor for the T2DM prognosis. These outcomes were in contrast to studies in India and Tunisia, where the most common genotype in T2DM patients was GG (37, 38). This evidence supports the assumption that TNF-α gene polymorphisms and their genetic and environmental aspects could be a significant risk factor for T2DM.

In Ethiopian patients, a significant relation was observed between TNF-α (−308) GG genotype gene polymorphism and T2DM. Contrary, there was no significant difference in the frequency of AG and AA genotypes (6).

In another study, INF-γ + 874 and TNF-α-308 gene polymorphisms in Egyptians were examined in relation to T2DM susceptibility. The genotypes of IFN-γ+874 (AA) and TNF-α-308 (AA) were significantly higher in T2DM patients comparing with healthy individuals (5).

Among TNF-α-308 genotypes, genotype AA was observed to be significantly related to a 4.3-fold enhanced risk for T2DM. The outcomes of a study about the relationship of TNF-a 308 G/A Polymorphism with T2DM in Iranian Kurdish people demonstrated that GA and AA genotypes were significantly related with 2.24- and 3.18-fold enhanced risk for T2DM, respectively (39).

Different researches have concentrated on the relationship between T2DM and TNF-α -308 G/A polymorphism and, but the outcomes are unknown. In these studies, the evidence showed that TNF-α -308 G/A polymorphism did not involve in T2DM progress. There are a few points that should be considered for contradictory outcomes in the initial reports. First, ethnic differences can be attributed to these distinct outcomes because the TNF-α -308 G/A polymorphism distributions are not the same among different ethnic populations. For example, the frequency of the TNF-α -308 G/A polymorphism allele varies from 9 percent in the Chinese population (40), 16% in Scandinavian and French populations (41, 42), 18% in German (43), to 24% in Australians (15). In addition, some environmental factors, small sample size, and study design may influence the outcomes. These differences may be because of genetic makeup differences among various research populations and variability of risk factors in their environment. Therefore, further researches in the various population are required to explain the exact role of TNF-α -308 G/A polymorphism in susceptibility to T2DM.

The present study examined only a single SNP, −308 G/A polymorphism and did just a single SNP analysis on the relation of this SNP with the susceptibility of T2DM.

There are several SNPs in the TNF promoter other than the −308 G/A polymorphism that may show crucial influence on the transcription of the TNF-α gene (44, 45). Therefore, other TNF-α promoter polymorphisms can be assessed.

As a result, genetic polymorphisms of IFN-γ (+874) and TNF-α (−308) are implicated in the genetic vulnerability for T2DM development in the Kurdish population. Polymorphism of TNF-α-308 AA and IFN-γ+874 TT and AT can be considered as a genetic biomarker for T2DM patients. Therefore, early screening of these two genetic polymorphisms may assist in the early control and management of T2DM.

Conflict of Interest

The authors declare that there is no conflict of interest.

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