Interleukin-6 (IL-6) Gene Polymorphism Patients with Type 2 Diabetes in Thi-Qar Province

Nasrallah Shamki Fayyad[1], Hassan Risan Al-Rikabi[2*]

Abstract

This study was conducted in the Biotechnology Research Unit, Mazaya University College, for the period from January to March 2021. This study includes 150 participants (100 T2DM patients and 50 healthy as a control group). Through the analysis of the results, it was found that the highest incidence of type 2 diabetes was in patients whose duration of illness ranged from (1-4) years and reached 70%, clear significant difference (P.Value = 0.00**). The results of the study analysis also showed that there was no significant difference between the two groups of patients and the comparison according to the interleukin levels, where the value of (P-value =0.41). The results of the analysis of the study through the analysis of genotypes and phenotypes showed that there was no significant difference between the two groups of patients and the comparison, where the value of (P.Value = 0.917). In this study, 25% of patients have GG genotype however 61% have GC genotype without significant difference between patients and control group (OR=1.10). While the frequency of the CC genotype was 14%, There is a clear significant difference between the group of patients and the comparison group (OR=1.56*). Mutations in the IL-6 gene were recorded in the gene bank with accession numbers. LC656471, LC656472, LC656473, LC656474, LC656475, LC656476, LC656477.

Key Words: IL-6, Type 2 Diabetes Mellitus, Thi-Qar Population.

DOI Number: 10.14704/nq.2022.20.4.NQ22113 **NeuroQuantology 2022; 20(4):227-232**

Introduction

Diabetes is one of the most common diseases in the world in developed and developing countries, affecting young and old, men and women. Scientific studies have shown that 8-5 percent of individuals have diabetes. Type 2 diabetes (T2DM) is an endocrine disease that is associated with often overweight or obese. It affects millions of people worldwide with a rapid increase in prevalence and incidence (Danaei *et al*, 2011) Rapid urbanization and increasingly sedentary lifestyles have increased the global prevalence of T2DM in recent decades. (Guariguata *et al*, 2014) A genetic polymorphism is a difference in the DNA sequence between individuals, groups or populations. The polymorphism may be the result of accidental processes, or it may be the result of external factors affected by the environment. One type of polymorphism is SNPs, which is the replacement of one nucleotide in a specific sequence in the human genome. The possibility of ethnic differences influenced the occurrence of IL-6 gene polymorphisms in European and Asian populations (karki *et al*, 2015; Mohammed and Qasim, 2021). Three million SNPs have been identified in humans, about one million of which are used to see if there is an association between the SNPs and susceptibility to diabetes and cancer.

Corresponding author: Hassan Risan Al-Rikabi

Address: [1]Biology Department, College of Education for Pure Science, University of Thi-Qar, Thi-Qar, Iraq; [2*]Biology Department, College of Education for Pure Science, University of Thi-Qar, Thi-Qar, Iraq.

[2*]E-mail: hassanalrekaby@utq.edu.iq

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 February 2022 **Accepted:** 23 March 2022

(Teama,. 2018). IL-6 is a multifunctional cytokine produced by a variety of cells such as endothelial cells and fat cells (and smooth muscle cells, fibroblasts, lymphocytes, and macrophages) (Barnes *et al*,. 2013). In obesity, enhanced production of IL-6 and other pro-inflammatory mediators as a result of a higher BMI is associated with insulin resistance (Xu *et al*,. 2003). Approximately 80% of patients with type 2 diabetes are overweight and maintaining glucose homeostasis depends on natural insulin secretion by pancreatic cells and normal insulin sensitivity (Cersosimo *et al*,. 2018; Bokov et al., 2022). It is estimated that (30-70%) of the risks of type 2 diabetes can be linked to genetics. Recently, studies have identified candidate cytokine genes involved in the pathogenesis of T2DM, one of which is (IL-6), and the production of this cytokine occurs through the expression of (IL-6) gene located on chromosome 7p15-21p (Banerjee & Saxena,. 2014).

Materials and Methods

Samples Collection

A blood sample of 100 samples was collected from the Diabetes and Endocrinology Center in Thi Qar Governorate for people with type 2 diabetes, and it represents the experiment group. And 50 blood samples for healthy people (students and self-employed people) Where a quantity of (2 ml) of venous blood was taken from the two groups of healthy and sick people, and the blood samples were placed in tubes containing the anticoagulant substance (EDTA) and kept at a temperature of 20°C. Also, (2) ml of blood was put into (Gel tube) tubes. For the purpose of isolating serum and quantifying IL-6 in patients and healthy subjects and comparing serum levels. An information form was approved for the two groups of patients and healthy people, including (age, smoking, area of residence, blood pressure, and family history).

DNA Extraction

DNA extraction from patient and healthy samples included several step based on the leaflet attached to kit DNA Extraction manufactured by Geneaid (Korean origin).

Polymerase Chain Reaction (PCR)

PCR technique was used to amplify the (IL-6) genes according to the method of work recorded (Arand *et al*., 1996).

Primers

PCR technique was used to amplify the (IL-6) gene using specific primers F-5-AACCTCCTCTAAGTGGGCTGA-3 and R-5-TGAGCCTCAGA CATCTCCAG-3 which design in this study to amplify 679bp.

Table 1. PCR Condition for amplification of gene IL-6

No.	Steps	Temprature	Time	No. of cycle	Size (bp)
1	Intial denaturation	95	10 m.	1	676
2	Denaturation	95	30 sec.	30	
	Annealing	59	30 sec.		
	Extention	72	35 sec.		
3	Final extention	72	10 m.	1	

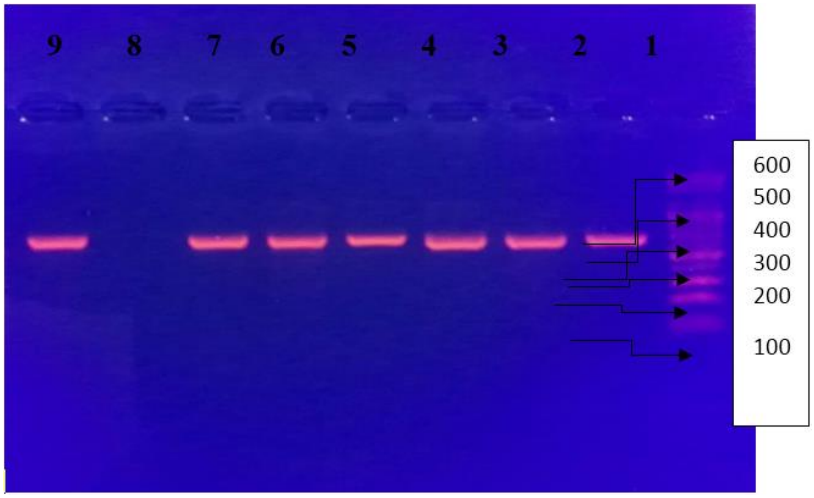

Figure 1. Show Electrophoresis of PCR products on 2% agarose gel. IL-6 gene and bundle appearance at the base pair 679bp

Measurement of IL-6 Levels in Serum

The measurement method (Kishimoto,. 1989) was used to measure the levels of (IL-6) in the serum based on the leaflet attached to kit (Korean origin).

Results

Table 2. Distribution of the patient group according to the duration of the injury

duration of injury (year)	Patient Group		P.value
	N	%	
4-1	70	70%	0.00**
9-5	18	18%	
10≥	12	12%	
Total	100	100%	
$^{2}\chi$= 4.33 df=1 Pvalue≥0.05			

Table 3. Distribution of control and patient groups according to IL-6. Level

Level IL-6	Control group (N=50)	Patient Group (N=100	T-Value	P.Value
IL-6	6.59±3.68	5.99 ± 3.14	-0.81	0.41

Table 4. Genotype and phenotypic frequency of the IL-6. gene

Genotype	Phenotype IL-6 Mean± SD	P.value
GG	6.53 ± 3.82	0.917
GC	6.61 ± 3.99	
CC	5.91 ± 2.48	
P.value ≥ 0.05		

Table 5. Frequency of IL-6 genotypes for both patient and comparison groups

genotypes	Control group(N%)=50	Patients group(N%=100)	OR	95% CI
GG	14(28%)	25(25%)	1.0	----------
GC	31(62%)	61(61%)	1.10	0.50-2.41
CC	5(10%)	14(14%)	1.56*	0.46-5.27
Total	50(100%)	100(100%)		
Allele frequency				
G	59(59%)	111(55.5%)	1.0	----------
C	41(41%)	89(44.5%)	1.15	0.70-1.87
Total	100(100%)	200(100%)		
OR: Odd Rations 95% CI Confidence Interval				

Table 6. Analysis of mutations in the IL-6 gene in people withT2DM

Mutation	Location	Location on the nuclotide	Type	F	Accession number
Deletion A732	promotor	7:22722620	Deletion	%1	LC656471
G>C 739	promotor	7:22726627	transversion	%1	LC656471
T>A 926	promotor	7:22726814	transversion	%1	LC656471
G>A 1053	promotor	7:22726941	transition	%1	LC656472
G>C 997	promotor	7:227268585	transversion	%1	LC656472
G>C 739	promotor	7:22726627	transversion	%1	LC656473
T>A 926	promotor	7:22726814	transition	%1	LC656473
T>A 927	promotor	7:22726815	transition	%1	LC656473
G>A 949	promotor	7:22726837	transition	%1	LC656473
G>C 955	promotor	7:22726843	transversion	%1	LC656473
T>A 958	promotor	7:22726846	transition	%1	LC656473
T>C 969	promotor	7:22726858	transition	%1	LC656473
Ins A 756	promotor	7:22726814	Insertion	%1	LC656474
T>A 926	promotor	7:22726814	transition	%1	LC656475
T>A 926	promotor	7:22726814	transition	%1	LC656476
C>G 1138	promotor	7:22727026	transversion	%1	LC656477

Discussion

The results of the current study based on the sequencing analysis of the (IL-6) -174C>G rs 1800795 gene revealed (15) point mutations in the group of patients with type 2 diabetes and (3) point mutations in the comparison group when compared with the reference sequence (NG_000007.14). The sequences containing these mutations were recorded in the National Center for Biotechnology Information (NCBI), the nitrogenous base adenine (A) in four sites, and the nitrogenous base adenine (A) was introduced in one site (756) and the nitrogenous base (G>C) was replaced in Five sites and the rule (T>A) was replaced in six sites. The base (C>G) is changed at one location.

The IL-6 single nucleotide polymorphism of the region (-174C>G rs1800795) is one of the functional polymorphisms of the gene in this region (-174C>G) located at the beginning of the main transcription site of the gene and the presence of any of the nitrogenous bases (C,G) It leads to the appearance of two different alleles of the gene, which leads to three possible genotypes (GG, GC, CC) (Zavaleta-Muñiz *et al,.* 2013; Huldani et al., 2022). Gene polymorphisms play key roles in regulating gene expression (Kamali-Sarvestani *et al,.* 2005). Polymorphisms of the IL-6 gene can affect circulating levels of interleukin (Stryjecki & Matich,. 2011). The region (Promotor -174) in the IL-6 gene has been shown to have an important biological function (Fishman *et al,.* 1998).

Studies of single nucleotide polymorphisms (SNPs) of the IL-6 gene in the Promotor region in different populations around the world have suggested a possible role for T2DM susceptibility as well as

insulin resistance and impaired glucose tolerance (Kristiansen & Mandrup-Poulsen,. 2005). The results of the current study showed when displaying the genotype distribution and frequency of alleles -174C>G (4880 NG_011640.1) for the gene (IL-6) in the control and patients' groups, where the frequency of the homozygous genotype (GG) in the group of patients was (25%) and without differences insignificant as the value of (OR=1.0) As for the frequency of the heterozygous genotype (GC) it was (61%) in the group of patients without significant differences, where the value was (OR = 1.10) value (OR=1.56*) As for the frequency of alleles, it was reached in the (G) allele in the group of patients (55.5%) and the (C) allele (44.5%) without significant significant differences. The results of the current study showed that there is a strong relationship between the duration of infection and type 2 diabetes, as it reached the highest infection rate in patients whose duration ranged between (1-4), reaching (70%), and the lowest infection rate in patients whose duration of infection ranged (≥10) and with a very high moral difference. They also agreed with the results of the study Cai *et al*. (2015), where duration of infection and other characteristics such as sample size and patients selected had a clear effect on nephropathy in patients with T2DM. It also agreed with the results of Medenilla *et al.* (2014) polymorphism The IL-6 gene increased blood sugar in pre-diabetes for Filipino patients with T2DM, where the mean age of the diabetic group was numerically higher than the control groups and pre-diabetes patients.

The results of the current study showed that there was no significant difference between the two groups of patients and the comparison and without a significant difference, where the value of (P = 0.41) differed from the results of the study of Bowker et al. (2020), which provided extensive human genetic evidence supporting the role of chronic inflammation through the pathway (IL -6) in T2DM. It also differed from the results of the study of Rodrigues et al. (2017), where the levels of (IL-6) were elevated in the patient group compared to the control group. Kristiansen & Mandrup-Poulsen (2005) indicated that IL-6 may have a direct effect on glucose homeostasis and metabolism or may act indirectly by acting on adipocytes and beta cells in the pancreas. In humans, this gene is linked to chromosome (7p15q21), where it was found that (IL-6) mRNA expression and insulin resistance have a significant relationship (Cardellini et al., 2005, Ansari et al., 2022).

Conclusion

The results of the study showed a relationship between mutations and polymorphisms in the gene IL-6 and type 2 diabetes mellitus and The polymorphisms, deletion and insertion mutations had an important role in the pathophysiology of type 2 diabetes mellitus. The study recorded some mutations in the Clinical Variation website at NCBI's National Center for Biotechnology Information. The current study also indicated that mutations affecting the encoded genes could be a direct cause of impaired insulin production in people with type 2 diabetes.

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