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Verifying The Association Between IL6 -174G/C Polymorphism in Type 2 Diabetes Mellitus

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Abstract Background : Diabetes mellitus, also known as blood sugar, is a series of metabolic disorders described by high blood glucose levels (hyperglycemia), low blood glucose (hypoglycemia), or both, resulting from defects in insulin production, insulin action, or both. Numerous studies have shown that interleukin (IL-6) acts on skeletal muscle cells , liver cells, and pancreas cells to influence glucose balance and metabolism, which directly or indirectly contributes to the development of diabetes. Research in this area is crucial because diabetes is recognized as a major risk factor for many diseases like Diabetic retinopathy, Diabetic nephropathy, Diabetic Neuropathy , heart disease and others. Patients and methods : In this study, we tested three of genotypes GG; GC ; CC for IL6 -174G/C polymorphism to determine its association with type 2 diabetes and its effect on insulin. One hundred T2DM patients and 100 clinically healthy participants were enrolled in the study. **Results and Conclusion** : Based on the results presented by the researcher, the three genetic polymorphisms GG, GC, and CC of the IL6-174G/C polymorphism did not appear to be associated with type 2 diabetes or determine the course of the disease. Given society's demand for radical solutions and the necessity of reducing the increasing number of deaths every year, these findings underscore the need for continued research into understanding the complex interplay of factors that contribute to diabetes-related complications.

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Keywords: *Diabetes, IL6 -174G/C polymorphism, PCR.*

1. INTRODUCTION

Diabetes, also known as mellitus, is a metabolic disease that is increasingly prevalent (1). Numerous studies indicate that the risk factors for metabolic syndrome, impaired glucose tolerance, and a combination of genetic factors and obesity contribute to an elevated likelihood of developing type 2 diabetes.(2)

The World Health Organization has classified diabetes as the most prevalence metabolic disease in the world, is mainly caused by two main factors the first is decreased insulin sensitivity in tissues As for the second decreased ability of pancreatic β-cells to release insulin (3). Many researchers have concluded that many people with type 2 diabetes can recover by preventing the disease and treating risk factors, even if there is a genetic cause for the disease (4,5). Epidemiological studies have shown genome-wide association that the disease is complexly polygenic. Most of these genetic loci increase the likelihood of developing type 2 diabetes by tampering with insulin action.(6,7,8)

Diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, heart disease, and others are the most serious complications of type 2 diabetes, and there is a large percentage of patients who suffer from these complications as a result of not following a healthy diet. Therefore, many studies recommend reducing the occurrence of These complications in patients with type 2 diabetes (9,10). This involves optimizing blood glucose levels according to personalized targets, reducing blood pressure, moderating protein intake in the diet, and managing weight.(11,12)

Many different types of cells, including immune cells, skeletal muscle cells, smooth muscle cells, and islet beta-cells, produce the protein interleukin (13,14). Interleukin-6 (IL-6) can be defined as a versatile pro-inflammatory cytokine (protein) that plays a major role in regulating immune and non-immune processes in all cells and tissues in the human body (14,15), In addition to protecting β-cells against cytokine-induced apoptosis, interleukin-6 (IL-6) can also produce insulitis (16). Type 2 diabetes risk factors may include the (GG) genotype or the -174 G variant within genes .(17)

Serious problems, and complications, may result from the worsening of type 2 diabetes due to an imbalance in immune-related systems. It may be possible to avoid or treat this dangerous condition and its complications if more is known about the processes linking inflammation to diabetes and related problems .(18)

Ultimately, the researcher presented in a sequential manner the relationship between three genetic variants and kidney failure, one of the complications of type 2 diabetes. The aim of this study is to diagnose the role of genetic variants in increasing patients' exposure to complications, with a focus on kidney failure, which is considered one of the most serious complications in patients with the Type 2 diabetes.

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2. METHODS

2.1 Samples collection

Samples, medical examination, and patient data were collected from the AL MUSTANSIRIYA UNIVERSITY NATIONAL DIABETES CENTER, Iraqi Diabetes Association in IRAQ BAGHDAD, Collecting the samples and required information took a period of 3 months, Considering patients' privacy and preserving their personal data, the exclusion and inclusion criteria were included according to the clinical condition. One hundred unrelated patients with Type 2 Diabetes Mellitus were included in the study. Patient selection was based on age (above 40), duration of diabetes (over 10 years), and the absence of other autoimmune diseases, chronic pancreatitis, and myocardial infarction. A control group of clinically healthy individuals (HC lot, n = 100) was matched with T2DM patients in terms of age and gender.

2.2 DNA Extraction

The Wizard Genomic DNA Purification Kit(19), is purposed for isolation of DNA from human serum samples (White blood cells),This kit has on a four-step process

1) 1-Lyses the cells and also the nuclei. For extraction of DNA from white blood cells, this step consists lysis of the red blood cells in the Cell Lysiss solution, than lysis of the white blood cells and their nuclei in the Nuclei Lysis solution.
2) 2-An RNase digestion step (it is optional for some applications.)
3) 3-Destroyed The cellular proteins by add salt precipitation for precipitates the proteins but leaves the high molecular weight genomic DNA in solution.
4) 4-Concentrated and desalted the genomic DNA by add isopropanol precipitation .(20)

2.3 Electrophoresis

Electrophoresis is the process of migration of charged molecules through solutions in an applied electric field. Specific Forms of Electrophoresis Commonly Used in Biochemistry is Paper Electrophoresis, new method of analytical, Gel Electrophoresis and the Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE). The gel used in the research is the Agarose. And Agarose gel electrophoresis is the principal technique used in In our research for determine the size of high-molecular-weight nucleic acids (DNA and RNA).(20)

2.4 Genomic DNA Isolation

We investigated the genomic DNA purification process using freshly drawn blood collected in EDTA, heparin, and citrate anticoagulant tubes. In the research, a PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) device was used, is a molecular biology method for finding DNA polymorphisms or genetic variations.The DNA manipulation and PCR-RFLP results showed no adverse effects. A 300-microliter blood sample was utilized for DNA extraction in each case (21). Based on my research, the key steps in genotyping the IL6 gene -174G/C polymorphism involve the extraction of genomic DNA, amplification of regions containing the polymorphisms, controlled temperature denaturation of amplicons, and denaturation in the presence of a fluorophore (22). The main stages of genotyping of IL6 gene -174G/C polymorphism are: extraction of genomic DNA, amplification of regions where polymorphisms are located, denaturation of amplicons under controlled temperature conditions and in the presence of a fluorophore. IL6 G-174G/C (rs1800795) was genotyped using the primers described in the literature. The primers used are:

F intern (G): 5′-GCACTTTTCCCCCTAGTTGT GTCTTCCG-3.
R intern (C): 5′-ATTGTGCAATGTGACGTCCTTTAGCTTG-3.
F extern: 5′-GACTTCAGCTTTACTCTTTGTCAAGACA-3.
R extern: 5′-GAATGAGCCTCAGACATCTCCAGTCCTA-3.

Table 1. *Program used for PCR amplification*

Number of cycles	Temperature (ºC) / time		
1X	94ºC /10 minut		
5x	94ºC /30 secund	67ºC /45 secund	72 ºC / 30 secund
38X	94ºC /30 secund	64 ºC /45 secund	72 ºC / 30 secund
1X			2 minut

The samples were analyzed in triplicate and the curves analyzed as the average of the three replicates. The reaction conditions for the IL-6 gene -174G/C polymorphism assay are shown schematically below (Table.1). The denaturation of the amplicons was performed thermally (94°C), and the renaturation was performed step by step at elevated temperature (94°C) at low temperature (4°C).

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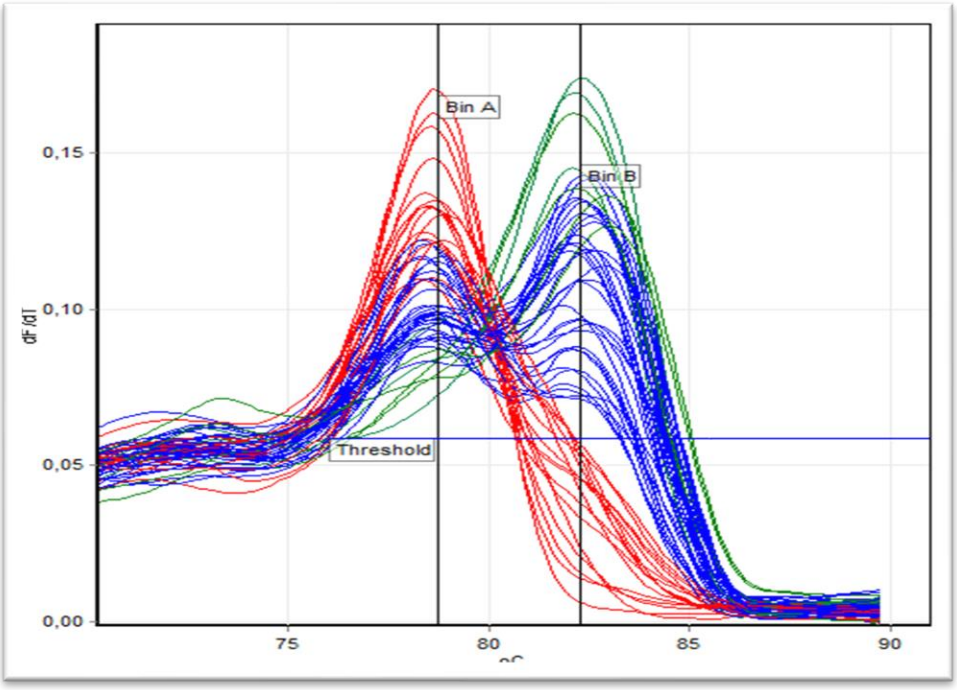

Figure 1 *Curves obtained by denaturing amplicons containing IL-6 G-174C polymorphism. The genotypes were set as follows: GG - red; GC - blue; CC – green.*

2.5 **Statistical Analysis**

The program known as the Wilcoxon signed-rank test, or The Wilcoxon test, was used to compare the variations in genotype and allele frequencies as well as clinical data between lots. corrected p value threshold was employed for correction of type I error.

3. RESULTS AND DISCUSSION

Depending on the statistical program (Wilcoxon test) the variations of the three polymorphisms in both the health control group (HC) and diabetes patients group (T2D) were found to be evaluatable. The Wilcoxon test, is used to evaluate the differences between two independent samples when sample sizes are small and the distributions are not regularly distributed. This non-parametric test is particularly useful when dealing with data that does not meet the assumptions of normality, providing a reliable means to evaluate differences between groups in such circumstances. The interleukin-6 gene may be associated with type 2 diabetes, as suggested by the statistical study. Individuals experiencing myocardial infarction, chronic pancreatitis, and autoimmune illnesses were excluded from the statistical analysis to prevent potential confounding effects. Table one contains a list of the subjects' attributes. The diabetic patients' body mass index (BMI) readings were discovered to be considerably the Wilcoxon test revealed that the values in the diabetic patients group were greater than those in the healh control group ($p < 0.02$). We conclude from this result that there is a significant difference between the two groups(diabetic patients group and healh control group), with the diabetes patients having statistically higher observed values for the specified variable. When comparing male and female subjects from the patient and control groups, similar increases in bady mass index (BMI) were observed. Additionally, the patients blood glucose levels, Cholesterol and Triglycerides levels were greater than those in the control group . In both groups, there were almost the same numbers of smokers and drinkers. Even when the respondents were divided into gender-specific groups, these results remained statistically significant.

Table 2. *Clinical and biochemical data of subjects selected for this study.*

Examined attributes	**T2D**		**HC**	
	Male	**Female**	**Male**	**Female**
Number	45	55	45	55
Age (years)	55.5 ± 5.0	54.2 ± 4.4	55.4 ± 4.7	54.3 ± 4.4
Duration of diabetes	7.3 ± 1.6	7.1 ± 1.7	(N.A)	(N.A)

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Body Mass Index	27.2 ± 1.5[a]	26.3 ± 1.5[a]	24.2 ± 0.5	22.7 ± 1.2
Hemoglobin A1c (%)	8.7 ± 0.5	8.6 ± 0.4	(N.A.)	(N.A.)
blood glucose	119.0 ± 16.6[a]	106.2 ± 15.6[a]	91.0 ± 7.5	92.0 ± 7.1
Cholesterol (mg/dl)	211.4 ± 35.2[a]	201.1 ± 27.7[a]	171.6 ± 16.5	169.7 ± 17.1
Triglycerides (mg/dl)	183.4 ± 65.6[a]	160 ± 56.4[a]	111 ± 24.3	111.6 ± 23.6
Smokers[#]	7	4	4	2
Drinkers[##]	5	0	4	0

1. T2D : type 2 diabetes.
2. HC : health control.
3 a : the P value for Wilcoxon test < 0.02.
4. N.A : not available; #: more than five cigarettes per day, for at least oyear.
5. ## : more than 5 units of alcohol per day, for at least one year.

Table 3. *presents the distribution of IL6 polymorphisms within the lots under investigation, providing a detailed breakdown of the genetic variations observed in the study cohort.*

Polymorphisms	**T2D**			**HC**		
	Male	**Femae**	**total**	**Mae**	**Female**	**total**
IL-6 GG	28	33	61	29	27	56
IL-6 GC	16	17	33	13	23	36
IL-6 CC	3	3	6	5	3	8
Hardy Weinberg equilibrium(X^2 test) (Wilcoxon test)	0.12	0.17	0.29	2.98	0.45	0.42

p value for GG= 0,048, p value for GC = 0,02, p value for CC = 0,02.

The distribution of IL-6 genotypes did not deviate from Hardy-Weinberg equilibrium. Even after correcting for gender stratification in the participants, the study did not uncover any significant link between IL6 gene -174G/C polymorphisms and type 2 diabetes. Therefore, from these data, we conclude that there is no relationship between the genetic variant IL-6 and the occurrence or development of type 2 diabetes in the population under study, based on the data and analysis performed.

Table4: *Association between type 2 diabetes and the IL6 gene -174G/C polymorphism in the investigated lots.*

Risk factor	**Male**	P value
GG	OR= 0.91, 95% CI: 0.4 -2.09	0.83
G	O.R.=1.74, 95%CI: 0.39-7.76	0.45
CC	O.R.=0.57, 95%CI: 0.12-2.54	0.45
C	O.R.=1.09, 95%CI: 0.47-2.5	p=0.83
	Female	

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GG	O.R.=1.58, 95%CI: 0.73-3.44	0.23
G	O.R.=1, 95%CI: 0.19- 5.19	1
CC	O.R.=1, 95%CI: 0.19- 5.19	1
C	O.R.=0.62, 95%CI: 0.29-1.36	0.23
	All subjects	
GG	O.R.= 1.22, 95%CI: 0.7 -2.15	0.4
G	O.R.= 1.36, 95%CI: 0.45- 4.08	0.57
CC	O.R =0.73, 95%CI: 0.24-2.19	0.57
C	O.R.=0.81, 95%CI: 0.46-1.42	0.4

NOTE "Lots" usually refers to the individual observations or data points in the matched samples under analysis when used in the context of the Wilcoxon test. These observations consist of the measurements or data gathered from every research subject and experimental unit

When data about additional features were tested in addition to the IL6 genotype, several variations were observed. Therefore, among males with T2DM, carriers of the GG genotype have a lower body mass index (BMI) than those with the IL6 C gene (Mann-Whitney U test < 0.05). Even after accounting for gender and age of diabetes development in the analysis, no significant association was identified between IL6 gene -174G/C and type 2 diabetes .

Although there is inconsistent evidence regarding the association of the development of type 2 diabetes with IL6 gene -174G/C polymorphism, there is an urgent need for further investigation and clarification in this area.(24)

It's possible that the link with type 2 diabetes (T2DM) is limited to slim male participants who carry the IL6 gene -174G/C or to overweight or obese subjects who carry the -174C allele. There is no evidence to support the links between IL6 gene -174G/C and BMI or T2DM.(25)

In the current investigation, IL6 gene -174G/C was not linked to type 2 diabetes, regardless of the inclusion of gender, BMI, or age of onset in the analysis. Discrepancies between the findings of this study and those already published may be attributed to factors such as the population's size and stratification, exposure to risk factors, variations in allele frequency within the population, and age range.(16,25,26)

Based on these findings, we may draw the conclusion that, in the opinion of the researcher, The above-mentioned examples confirm that interleukin-6 (IL-6) forms are not associated with type 2 diabetes, which leads to kidney failure. Researchers may have assessed various factors, including IL-6 levels or genetic variants related to IL-6, and concluded that IL-6 does not play a significant role in this particular aspect of the disease pathway. Given society's demand for radical solutions and the imperative to reduce the increasing number of deaths each year, these findings underscore the need for continued research in understanding the complex interplay of factors contributing to diabetes-related complications.

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