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ROLE OF TRANSCRIPTION FACTOR 7 LIKE RS7903146 AND RS12255372 GENE POLYMORPHISMS AND SELECTIVE BIOCHEMICAL TESTS IN TYPE II IRAQI DIABETIC PATIENTS

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HAYDER DHYAA KHALEEL ALHWAIDI

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May 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

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Master of Science in Chemistry

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Recent studies have related to see whether the TCF7L2 gene polymorphisms rs7903146 (C/T) and rs12255372 (G/T) are linked to the risk of developing T2DM in the Iraqi population. In this study, biochemical and genetic parameters. Real-time PCR was used to genotype the samples. In both patients and controls, the frequency of genotypes, alleles, anthropometric measurements, glycemia, and glycated hemoglobin (HbA1c) was measured. As result, The TCF7L2 SNPs rs7903146 and rs12255372 had genotyping success rates of 98.55 and 97.42 percent, respectively. For both SNPs, the allele and genotype frequencies were in Hardy-Weinberg equilibrium. Between patients and controls, the genotype and allele frequencies for (TCF7L2 SNP rs7903146) allele were not substantially different. The frequency of the (rs7903146 T) allele in the controls was 29 percent, whereas it was 28 percent in the patients (P = 0.61). The TCF7L2 SNP rs12255372 genotypic and allelic frequencies did not vary substantially between patients and controls. In controls, (rs12255372 T) allele frequency was 21%, but in patients, it was 27% (P = 0.42).

2022, 31 pages

Keywords: Type 2 diabetes, Genetic association, Transcription factor 7-like 2 (TCF7L2), Polymorphism

ÖZET

TİP II IRAK DİYABETİK HASTALARDA RS7903146 VE RS12255372 GİBİ TRANSKRİPSİYON FAKTÖRÜ 7'NİN ROLÜ VE SEÇİCİ BİYOKİMYASAL TESTLER

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Çalışmamız, TCF7L2 gen polimorfizmleri rs7903146 (C/T) ve rs12255372 (G/T)'nin Irak popülasyonunda T2DM geliştirme riski ile bağlantılı olup olmadığını görmekle ilgili olarak kurgulanmıştır. Bu çalışmada, biyokimyasal ve genetik parametrelerin incelenmesi için gerçek zamanlı PCR kullanılmıştır. Hem hastalarda hem de kontrollerde genotiplerin, alellerin, antropometrik ölçümlerin, gliseminin ve glikolize hemoglobinin (HbA1c) sıklığı ölçülmüştür. Sonuç olarak, TCF7L2 SNP'leri rs7903146 ve rs12255372'nin genotiplenmesi için başarı oranları sırasıyla %98.55 ve %97.42 olarak tespit edilmiştir. Alel ve genotip frekansları, her iki SNP için de Hardy-Weinberg dengesinde olarak tespit edilmiştir. TCF7L2 SNP rs7903146 aleli için genotip ve alel frekanslarının, hastalar ve kontroller arasında önemli ölçüde farklılık göstermediği rs7903146 T allel sıklığı kontroller için %29 iken hastalarda %28 olduğu (P = 0.61) tespit edilmiştir. TCF7L2 SNP rs12255372 için genotipik ve alelik frekanslar, hastalar ve kontroller arasında önemli ölçüde farklılık göstermemiştir. Kontrollerde rs12255372 T allel sıklığı %21 iken, hastalarda %27 (P = 0.42) olarak tespit edilmiştir.

2022, 31 sayfa

Anahtar Kelimeler: Tip 2 diyabet, Genetik ilişki, Transkripsiyon faktörü 7-like 2 (TCF7L2), Polimorfizm

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Hayder Dhyaa Khaleel ALHWAIDI

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CONTENTS

LIST OF SYMBOLS

°C	Degrees Celsius
µL	Microliter
±	Plus-minus
Bp	Base pair
dL	Deciliter
g	Gram
kb	Kilobyte
%	Percent
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliters
mmHg	Millimeter of mercury
ng	Nanogram

LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
AER	Albumin excretion rate
APC	Adenomatous polyosis coli
ARB	Angiotensin receptor blockers
BCL	B-cell lymphoma
BMI	Body mass index
BP	Blood pressure
CGC	Cancer gene census
DN	Diabetic neuropathy
DNA	Deoxyribonucleic acid
GDM	Gestational diabetes mellitus
HDL	High density lipoprotein
HLA	Human leukocyte antigen
LADA	Latent autoimmune diabetes in the adult
LD	Linkage disequilibrium
LDL	Low density lipoprotein
MCP-1	Monocyte chemoattractant protein-1
MODY	Maturity-onset diabetes of the young
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RIA	Radioimmunoassay
SD	Standard deviation
SNPs	Single nucleotide polymorphisms
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TCF7L2	Transcription factor 7-like 2
TNF	Tumor necrosis factor
WHO	World health organization

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Hyperglycemia is the primary symptom of type 2 diabetes, which is a metabolic illness with several contributing factors. Inadequate insulin production or resistance to insulin that has been secreted both play critical roles in the pathogenesis of type two diabetes (Lin *et al*. 2010, Kyrou *et al*. 2010). In addition to this, it may lead to a number of consequences, the most common of which are cardiovascular and endothelial illnesses (Ali *et al*. 2013, Desouza *et al*. 2009). An examination of the whole genome indicated that numerous genes have a role in the etiology of type two diabetes (Elbein *et al*. 2009). In particular, the transcription factor (7-like 2), or TCF7L2, gene is recognized as the best potential gene involved in everything from the impairment of insulin synthesis to the development of type 2 diabetes (Elbein *et al*. 2009). The transcription factor 7 like 2 (TCF7L2) gene is an essential component of the Wnt signaling pathway. It is an entero-endocrine transcription factor that is located on chromosome 10q (Castrop *et al*. 1992). Following this, stimulation of Wnt catenin promotes the assembly of -catenin with BCL9, which is then followed by translocation, into the nucleus and the formation of an active form with (TCF7L2) (Yi *et al*. 2005, Klaus *et al*. 2008). Activation of Wnt target genes, which are involved in cell proliferation, apoptosis, and tissue invasion, is the consequence of the complex (Zhang *et al*. 2008). The (TCF7L2) gene plays a significant role in type two diabetes by controlling the processes of adipogenesis, myogenesis, and pancreatic island formation. It modulates the production of a protein that is involved in the exocytosis of insulin granules, and it has an influence on the activity of (beta-cells) and granules that are responsible for the secretion of insulin (Xavier *et al*. 2009).

Trying to figure out what caused a linkage signal that had been found in chromosome 10's long arm to be discovered in the first place (Grant *et al*. 2006) data from Icelandic adults revealed a substantial correlation between type 2 diabetes (T2D) and (DG10S478) microsatellite marker. In addition, these findings were confirmed by the authors in cohorts consisting entirely of Caucasian people from both North America and Denmark. A tetranucleotide repeat known as the (DG10S478) microsatellite is responsible for, has six different alleles. Its found in a well-defined 92.1-kilobase linkage disequilibrium block

that is made up of exon 4 as well as a part of 2 large intronic sections that surround (TCF7L2) gene.

TCF7L2 is a transcription factor that's been previously associated with maintaining normal levels of blood glucose. It also plays a significant part in the Wnt signaling pathway. It is located on chromosome (10q25.3) and takes up a total of 215.9 kb (Yi *et al*. 2005, Duval *et al*. 2000). According to the (Lyssenko *et al*. 2007), It is most probable that abnormal regulation of the CGC gene, which is the gene that makes glucagon, When the TCF7L2 gene's expression and function alter, it's to blame for the pancreatic islets' poor functioning. As a consequence of this, insulin secretion may decrease, which in turn increases the chance of developing type two diabetes. The noncoding (SNPs) rs7903146 (C/T) as well as rs12255372 (G/T) found in the TCF7L2 gene are in substantial linkage disequilibrium with (DG10S478) microsatellite and indicate a strong connection with type 2 diabetes (Florez *et al*. 2006, Grant *et al*. 2006).

It has been observed that the (TCF7L2) SNPs rs 7903146 and rs 12255372 are associated with reduced insulin production and an increased risk for type 2 diabetes among persons living in North America who are unable to tolerate glucose. These authors suggested that the two different variations be genotyped in every study so that these findings could be verified.

TCF7L2 SNPs rs7903146 and/or rs12255372 have been linked to type two diabetes in a number of different populations from countries all over the world, including Finland, Tunisia, Poland, Germany, France, Iran, England, Japan, Sweden, China, and India, according to the results of studies that were conducted independently (Groves *et al*. 2006, Cauchi *et al*. 2006, Palizban *et al*. 2012, Horikoshi *et al*. 2007, Mayans *et al*. 2007, Saxena *et al*. 2006, van Vliet-Ostaptchouk *et al*. 2007, Ezzidi *et al*. 2009, Gupta *et al*. 2010, Mayans *et al*. 2007, Scott *et al*. 2006, Wen *et al*. 2010).

However, among populations from China, United Arab Emirates, and the Saudi Arabia, inconsistent findings have been observed (Saadi *et al*. 2008, Alsmadi *et al*. 2008, Guo *et al*. 2007, Ren *et al*. 2008).

1.1 Objectives of Study

The aim of this research was to see whether (TCF7L2) polymorphisms are linked to T2DM in Iraqi individuals. The following target was proposed :-

1. Determination of (rs12255372 and TCF7L2 SNPs rs7903146) gene polymorphism by using the (RFLP PCR).
2. Investigate the levels of biochemical markers in serum of patients with T2D by using ELISA assay and auto-analyzer.

2. LITERATURE REVIEW

(Bene *et al*. 2018) A disturbance in energy metabolism is a result of diabetes, which is caused by an inadequate amount of insulin in the body. This is mirrored in endogenous processes such as the carnitine cycle, which is an essential component of the beta-oxidation of fatty acids that occurs in the mitochondria.

(Karamanou *et al*. 2016, Bolodeoku and Donaldson 1996) Hyperglycemia, commonly known as diabetes mellitus, is a chronic disorder that is characterized by high blood sugar levels. Diabetes mellitus, or just diabetes, is another name for this ailment. Diabetes is named after the disease's excessive urination, and mellitus, which means "sweetened by honey" in Latin, alludes to the sugars in the diabetic's urine. Even the ancient Egyptians employed this sweet taste as a diagnostic tool. Thomas Willis discovered in 1674 that the urine of his diabetic patients had a sweet taste. One hundred years later, Matthew Dobson determined that sugar was the cause of the sweet taste, and that it was preceded and followed by sugar in the blood. Both of these discoveries are considered to be the foundation for the modern understanding of how diabetes is caused. Even though diabetes is described as a condition that is characterized by high blood sugar levels, fast and easy tests called dipstick tests are often used to detect glucose leakage into urine. This is because diabetes is classified as a disease that is characterized by high levels of blood sugar (Urologic Nursing. Jan/Feb2016).

The term "diabetes mellitus" refers to a groups of diseases that affect the body and are characterized by high blood sugar levels (hyperglycemia) and are brought on by insulin resistance, inadequate insulin synthesis, or high plasma levels. The autoimmune condition known as type one diabetes is characterized by the annihilation of (beta-cells) in the pancreas. Illness known as type two diabetes (T2D) is characterized by gradually deteriorating glucose control and is brought on by a dysfunctional pancreatic beta-cell population in conjunction with insulin resistance (Anık *et al*. 2015).

It is possible that defects in the function of beta cells might be traced back to mutations in a single gene (referred to as monogenic). Maturity-onset diabetes of the young, also

known as (MODY), is a collection of monogenic illnesses that are characterized by an autosomal dominantly inherited noninsulin-dependent form of diabetes that typically presents itself in adolescence or young adulthood prior to the age of 25. MODY is a rare form of diabetes that only affects 1% of individuals and is often misdiagnosed as T1DM or T2DM. MODY is characterized by a lack of insulin production in the body. Patients with MODY do not suffer from obesity or any of the other risk factors associated with T2DM, like as abnormal blood fat levels or high blood pressure (Guariguata *et al*. 2014).

Insulin is a hormone that is created by the (beta cells) in the pancreas. Insulin is the most important hormone when it comes to controlling the amount of glucose in the blood. The hormone increases the intake of glucose from the blood in the fat tissue and muscle, the liver and muscle cells store glucose as glycogen, and the uptake of fatty acids and their esterification in adipocytes. In addition, insulin prevents the breakdown of proteins, the hydrolysis of triglycerides, and the creation of glucose from glycerol, lactate, and, amino acids. Glucagon, a hormone that is likewise released by the endocrine pancreas, functions in a manner that is diametrically opposed to that of insulin. As a result of the hormone's action, the liver's glycogen stores are converted into glucose, which leads to an increase in blood sugar. In addition to this, glucagon stimulates the release of insulin, which enables organs that are reliant on insulin to use glucose. As a consequence of this, glucagon and insulin coordinate their activities in order to keep the amount of glucose in the blood at a healthy level.

In order for type 2 diabetes to develop, there must be a disruption in the normal, balanced relationship between insulin action and release. The majority of persons who develop type 2 diabetes are unable to boost their insulin production enough to make up for the insulin resistance they already have. Insulin resistance, on the other hand, is linked to being overweight, the ability of pancreatic cells to perform their functions steadily diminishes with age, even before the onset of clinical hyperglycemia. For these two faults, several mechanisms have been proposed (Charnogursky *et al*. 2014).

Diabetes mellitus-related neuropathies impact 60-70 percent of diabetic patients. Peripheral polyneuropathies, mononeuropathies, and autonomic neuropathies are

examples. Controlling blood sugar, cholesterol, and hypertension, as well as quitting smoking, can help to delay the start and progression of these neuropathies. We do not have effective drugs to address the pathophysiologic causes of diabetic neuropathies, except from controlling the risk factors listed above. Treatment is confined to relieving pain and addressing the neuropathic processes' end organ effects..

According to the (WHO), Two fasting glucose measurements above 126 are deemed diagnostic for D M, according to the current definition. People are considered to have impaired fasting glucose if their glucose levels range from 110 to 125 mg/dl. There are approximately 350 million people who suffer from diabetes mellitus (DM), and by the year 2030, diabetes will become the seventh leading cause of death worldwide. Additionally, the number of deaths caused by diabetes is expected to increase by 50 percent over the next 10 years (IDF Diabetes Atlas. 5th edition, 2012).

Number of people diagnosed with diabetes has increased over the world. However, the majority of those affected by the condition reside in nations with poor or moderate incomes, and approximately half of those who have diabetes are oblivious to the fact that they have the ailment (ADA, 2015).

One of the most prevalent medical problems of pregnancy is gestational diabetes mellitus (GDM) (Tieu and colleagues, 2014). Although GDM normally disappears after birth, it is linked to considerable perinatal and long-term morbidity for the mother and her child (Nankervis *et al*. 2010).

glucose intolerance of any degree before the start of pregnancy or upon first becoming aware of it throughout pregnancy. This includes undiagnosed diabetes mellitus discovered during pregnancy for the first time (Löbner and colleagues, 2006). This type is usually discovered between the (24th) and (28th) week of pregnancy. Women who have experienced GDM are more likely to develop diabetes later in life (Boney *et al*. 2005). In addition, children born to mothers who have GDM have a higher likelihood of becoming obese as children and as adults, as well as a higher likelihood of being unable to tolerate glucose (Alhyas *et al*. 2012). Diabetes mellitus, abbreviated as DM, is a metabolic

disorder that lasts for a long time and is characterized by elevated levels of glucose in the blood. This illness may be brought on by insufficient insulin synthesis, impaired insulin action, or both (Ashcroft and Rorsman 2012).

People who are at risk of developing (T2DM) have a higher than normal insulin resistance in their muscles, and this is the first sign of the disease. In addition, research has shown that beta-cell activity must be dysfunctional prior to the development of hyperglycemia. These alterations begin to manifest themselves around 10 years prior to diagnosis of diabetes (Ashcroft and Rorsman, 2012).

Insulin resistance is linked to changes in the pattern of lipids seen in the plasma. This leads one to believe that elevated glucose levels aren't the sole factor related with type 2 diabetes; a lipid profile may also be able to anticipate when the disease will manifest itself (Eckel *et al*. 2011).

The etiology of type two diabetes is not completely known; nevertheless, it is hypothesized that type two diabetes develops as a result of the interaction of a diabetogenic lifestyle (i.e. excessive caloric intake, insufficient caloric expenditure, and obesity) with a vulnerable genotype. Patients who are overweight are more likely to acquire type two diabetes (Hectors *et al*. 2011). On top of that, it has been hypothesized that environmental contaminants may be to blame in some instances of type two diabetes (McCarthy *et al*. 2010).

T2DM is commonly, but not always, related with metabolic abnormalities such as obesity, which may itself beget insulin resistance and lead to conditions in which blood glucose levels are raised. However, obesity is frequently connected with T2DM. People who have T2DM, in contrast to those who have type 1 diabetes, are not totally reliant on exogenous insulin, but they may by need to take insulin to control their hyperglycemia if it can't be managed with diet alone or with oral hypoglycemic medications. At least fifty inheritable variations have been documented to have an effect on susceptibility to develop type two diabetes. Type two diabetes is strongly associated with the home environment (Pociot *et al*. 2010).

People who have type 1 diabetes are usually insulin-dependent, and they have to take insulin injections many times throughout the day in order to keep their blood glucose level under control and to keep themselves alive. In addition, type 1 diabetes, and particularly the autoimmune process, is mostly the result of genetic variance (González *et al*. 2009). Over ninety percent of people who are diagnosed with diabetes have T2DM mellitus. This makes T2DM the most prevalent form of the disease (Barrett *et al*. 2009). There is a significant link between type 1 diabetes (T1D) genes and human leukocyte antigen (HLA) genes. Additionally, HLA variations confer a high risk of developing the complaint or a protection against it. There are currently around 40 inheritable loci that have been demonstrated to impact the risk of developing T1DM (Nathan *et al*. 2009).

Where the clock begins ticking for microvascular risk when hyperglycemia first appears, and where the clock starts ticking for macrovascular risk at some point prior to that, namely when insulin resistance first appears. It is widely agreed upon that patients with diabetes mellitus who have their blood sugar properly under control are far less likely to experience long-term consequences and those that do occur tend to be of a milder severity (Taubes *et al*. 2008). However, the results of some recent clinical trials call into question the idea that hyperglycemia is the primary factor in diabetic complications. These trials were successful in reducing blood sugar levels in patients with T2DM, but they failed to reduce deaths caused by cardiovascular disease (Milicevic *et al*. 2008).

The various processes by which diabetes causes these consequences include hyperglycemia and both functional and structural abnormalities of tiny blood arteries. This series of events is further accelerated by variables including excessive cholesterol, smoking, obesity, high blood pressure, and a lack of regular exercise. Although these mechanisms are not completely understood, they are involved in the chain of events (Thauland *et al*. 2008).

In contrast, the adaptive immune system, consisting of B- and T-lymphocytes and natural killer cells, is designed for antigen-specific recognition based on immunological memory, improving the speed and quality of the immune response upon re-exposure to the same antigen. The complex interplay between innate and the adaptive immune responses and

the tight interaction between APC and T- and B lymphocytes is still not fully understood but intensely investigated.

It is well known that the adaptive and innate immune systems are interacting closely, and they have been shown to influence their function and phenotype by specific cytokine signalling (Kintscher *et al*. 2008) how T cells, as mediators of the adaptive immune system, are involved in macrophage activation and recruitment into adipose tissue is still not well understood. A very recently published human and animal study suggests that insulin resistance and adipose tissue infiltration by (CD4+ T cells) is a primary events leading to subsequent recruitment of macrophages (Lara-Castro and Garvey 2008).

Patients with type two diabetes, particularly those who also have nephropathy, have a distinct lipid pattern that differs from that of non-diabetic individuals. This lipid pattern is distinguished by a buildup of triglycerides in the muscles and the liver, both of which are located in the body, a reduction in high density lipoprotein (HDL) concentrations, and cholesterol levels that remain the same in low density lipoprotein (LDL) (Van Deutekom *et al*. 2008).

One possible approach is to consider more in terms of a disease continuum, with the traditional autoimmune type o diabetes on one end of the continuum and the conventional metabolic (T2DM) on the other.

However, the current practical categorization rules continue to adhere to the conventional idea that type one diabetes (T1DM) and type two diabetes (T2DM) are two separate illnesses that may be attributed t wholly separate, if still mostly unknown, causes.(Baptiste-Roberts *et al*. 2007).

There is a higher prevalence of diabetes in African Americans, Hispanic Americans, and Native Americans than there is in Caucasian Americans. Risk factors for developing diabetes include having a family history of the disease, being inactive physically, having high blood pressure, and not being physically active. As was noted earlier, obesity is

another prevalent factor that contributes to insulin resistance and increases the chance of getting diabetes (Ueki *et al*. 2006).

After a meal, the amount of glucose that is present in the blood often rises, which in turn causes the -cells that are located in the "Islets of Langerhans" in the pancreatic gland, which is the location where insulin is created, to release some of their stored supply of the hormone. Insulin works by stimulating muscle and fat cells to extract glucose from the circulation via a process called glycolysis. It also works by stimulating the liver to digest glucose and store it as glycogen through a process called glycogenesis. In this manner, the glucose concentration in the blood is brought back down to normal levels.

Indeed, the authors could demonstrate that macrophage infiltration occurs only after 8 weeks of high fat feeding, in contrast to the much earlier development of insulin resistance and T cell infiltration into the adipose tissue, which both occurred already after 3 weeks of overfeeding. Based on these findings, it appears that insulin resistance and T cell activation are the major processes that occur before macrophage infiltration, and that macrophages only contribute to insulin resistance in a later stage through the increased release of cytokines (Fourlanos *et al*. 2005).

One of the most prevalent endocrine and metabolic diseases affecting children is type 1 diabetes, which also moves quite quickly through its stages. LADA, is a kind of diabetes that develops slowly over time and is distinguished from T1DM by the presence of diabetes-associated autoantibodies and insulin independence at the time of diagnosis.

The death of insulin-producing cells in the pancreas, which leads to total insulin shortage and T1DM, is the root cause of (T1DM). This damage is largely brought on by an autoimmune reaction. There are certain gaps in our knowledge on why this takes place. The condition can manifest itself at any age, although it is most common during adolescence and childhood. In general, it can be diagnosed at any age.

3. MATERIALS AND METHODS

3.1 Material

3.1.1 Marker and primers

In (Table 3.1)The DNA ladder and primers that were utilized in this research may be found.

Table 3.1 Marker and primers

NO.	MARKER	MANUFACTURING COMPANY	ORIGIN
1	Primers	Bioneer	Korea
2	100 bp DNA ladder	Intronbiotechnology	Korea
3	50 bp DNA ladder	Intronbiotechnology	Korea

3.1.2 Primers

TNF-α gene (TNFA -308 G/A (rs1800629) and MCP-1 (-2518G/A) gene polymorphism primers were designed by Wilson et al. 1992, and Simeoni et al. 2004.These primers were provided from (GIBCO BRL Grand Island, NY, USA-Qiagen, Mississauga, Ontario, Canada) as following Table 3.2:

Table 3.2 The primers for the multiplex PCR, together with their respective sequences and amplicon sizes

PRIMER	SEQUENCE		AMPLICON	
rs7903146	F	5'-ACAATTAGAGAGCTAAGCACTT TTT AGGTA-3'	188-bp	NEB, USA
	R	5-GTGAAGTGCCCAAGCTTCTC-3'		
rs12255372,	F	5'-GAGGTGTACTGGAAACTAAGGC-3'	226-bp	Thermo Scientific, USA
	R	5'-GAGGCTGAATCTGGCACTCA-3'		

3.1.3 Restriction enzyme

Table 3.3 includes a list of the restriction enzymes that were utilized in the RFLP-PCR experiment, along with the business that manufactured them and their place of origin.

Table 3.3 Restriction enzyme

RESTRICTION ENZYMES	SNP	RESTRICTION SITE	COMPANY/COUNTRY
RsaI enzyme	rs7903146	5'-GT▼AC-3'	GIBCO BRL Grand Island, NY, USA. Qiagen, Mississauga, Ontario, Canada.
2 U Tsp509I enzyme	rs12255372	5'-▼AATT-3'	

3.2 Methods

3.2.1 Collection of samples

A diagnosis of diabetic nephropathy, also known as microalbuminuria, was made when the (AER), as determined by (RIA), had a concentration of between 30 and 300 mg/24h in at least two of the three 24-hour urine collections, that were performed over the course of three months. When we first started out with the research, 50 diabetic cases with nephropathy as well as For the purpose of the research, fifty diabetic patients who did not have nephropathy were included. However, 5 cases with nephropathy, Among those instances, there were five cases that did not have nephritis. For a variety of reasons, those individuals were eliminated from the research. In total, 100 diabetic cases were used in the study. There are no patients in the study group, whether they have nephropathy or not, who are receiving therapy with ARB or ACE. The participants in this study included 90 diabetic rehabilitants and a healthy control group consisting of 100 individuals. The diabetic rehabilitants were given exercise therapy to improve their kidney function. The people who were given the 75-g oral glucose test at 0 and 2 hours are the ones who make up the control group. The results of the test showed that they had normal glucose levels. All of the performers provided their informed consent for the action. It was necessary to

obtain a comprehensive medical history for each patient. It was possible to establish phenotypic features. Age, gender, and blood pressure were all taken down.

3.2.2 Blood samples

After using alcohol 70 to castrate the region of aspiration of blood from the cubital fossa tone, a blood sample measuring 5 milliliters was taken from each of the cases as well as the control groups. The sample that was taken was carefully placed in the following two tubes in the following order:

A. Two milliliters (2 ml) of blood in a five milliliter (EDTA tube) tube to be used for WBCs attention and PCR method to be utilized for detection of (TCF7L2 SNPs rs7903146 and rs12255372) gene polymorphism.
B. Three milliliters (ml) of blood were placed in a plain tube, often known as a serum tube. The blood samples were then centrifuged at 4700 revolutions per minute (RPM) for five minutes (to acquire blood serum), and the blood samples were also solidified at -80 degrees Celsius for the inclusion of TNF-a cytokine and biochemical labels.

3.2.3 Molecular methods

Genomic DNA extraction: The Accupower Genomic DNA extraction kit (Whole Blood) Geneaid USA was used in order to isolate genomic DNA from blood samples.

Genomic DNA Profiling: The genomic (DNA) obtained from blood samples was analyzed with a spectrophotometer manufactured by Thermo Scientific in the United States called a Nanodrop spectrophotometer. This instrument checks and measures the purity of DNA by measuring the absorbance in at (260/280 nm).

RFLP-PCR Technique: The (RFLP-PCR(technique was performed for detection (TCF7L2 SNPs rs7903146 and rs12255372)gene gene variation in patients' blood samples as well as control blood samples from healthy individuals.

PCR master mix preparation: The (PCR master mix) was created by utilizing an AccuPower PCR PreMix Kit, and the preparation of this master mix was done in accordance with the instructions provided by the business, which are detailed in (Table 3.4) as show.

Table 3.4 Mixture of PCR

MIXTURE SOLUTION	VOLUME	CONCENTRATION
Forward primer	2.5µL of each primer	10pm / µl
Nuclease free water	40µL	-
Master mix	PreMix	1X
Reverse primers	2.5µL of each primer	10pm / µl
Target DNA	5µL	20 ng / µL
Total volume	50µL	-

After that, the components of the PCR master mix that were previously indicated in the table above were put into a standard AccuPower PCR PreMix Kit. This kit includes all of the additional components that are required for a PCR reaction, such as primers, probes, and enzymes (Taq DNA polymerase, Tris-HCl pH: 9.0, loading dye, KCl, dNTPs, MgCl2, and stabilizer). After that, each PCR tube was placed in its own Exispin vortex centrifuge and spun at a speed of 3000 rpm for three minutes. The sample was then put into a PCR thermocycler (Mygene. Korea).

PCR Program: This program was listed in Table 3.5, 3.6.

Table 3.5 Amplification conditions of (TCF7L2 SNPs rs7903146)

STEPS	TEMPERATURE	TIME	NO. OF CYCLES
Final elongation	72 oC	5 min	1 cycle
Annealing	60oC	30 sec	35 cycle
Denaturation	94 oC	30 sec	
Elongation	72 oC	45 sec	
Hold	4 oC	forever	-
Initial denaturation	94 oC	2 min	1 cycle

Table 3.6 Amplification conditions of (TCF7L2 SNPs rs12255372) gene

STEPS	TEMPERATURE	TIME	NO. OF CYCLES
Final elongation	72 oC	10min	1 cycle
Elongation	72 oC	1.5 min	35 cycle
Annealing	55oC	1 min	
Denaturation	94 oC	1 min	
Hold	4 oC	For ever	-
Initial denaturation	94 oC	5min	1 cycle

Examination of PCR products The following procedures were taken in the analysis of PCR products using agarose gel electrophoresis:

1. An agarose gel with a concentration of 1% was manufactured by dissolving (1X TBE) in a water bath maintained at (100°C) for 15 m. The gel was then allowed to cool to 50°C.
2. After that, three microliters of a stain made of ethidium bromide was added to the agarose gel solution.
3. After ensuring that the comb was in the correct position, the tray was given the agarose gel solution to be put into it. After that, it was left to re-establish its consistency at room temperature for fifteen minutes. After that, After gently removing the comb from the tray, 10 ul of a (100 bp DNA Ladder) was applied to the first well of the plate.
4. A 1X TBE buffer it was added to the electrophoresis chamber once the gel tray had been secured there. After that, an electric current of 100 volts and 80 AM was carried out for a whole hour.
5. 5. The PCR products were seen with the help of a UV trans illuminator and shot with a digital camera (Sambrook and Russel, 2001).

Preparation of the RFLP-PCR mix The RFLP-PCR mix was made by utilizing 2 units of the RsaI restriction enzyme and 2 units of the Tsp509I restriction enzyme for the (TCF7L2 SNPs rs7903146 and rs12255372) genes polymorphism, respectively.

These reaction mixes were carried out independently in accordance with the instructions provided by the manufacturer, in Table 3.7.

Table 3.7 Mixture for genotype (TCF7L2 SNPs rs7903146 and rs12255372)genes

MIXTURE SOLUTION	VOLUME
Restriction enzyme (10 unit)	1 µL
Restriction enzyme buffer 10X	2 µL
Total volume	2 µL
Free nuclease water	7 µL
PCR product	10 µL

Following that, the master mixture was put into an Exispin vortex centrifuge, at 3000 rpm for two minutes, and then it was moved into an incubation chamber set at 37 degrees Celsius for the night. After that, the (REFLP-PCR) product was evaluated using agarose gel electrophoresis (2.5 percent), and the results of the analysis are shown in Table 3.8 and Figures 3.1, 3.2, 3.3, and 3.4 as follows:

Table 3.8 Restriction enzymes as well as the size of DNA pieces

RESTRICTION ENZYMES	GENE	GENOTYPE	FRAGMENT SIZE
2 U RsaI	(TCF7L2 SNPs rs7903146)	CC	159- and 29-bp
		CT	188-159- and 29-bp
		TT	188 bp
Tsp509I	(TCF7L2 SNPs rs12255372)	GG	151 and 75 bp
		GC	226, 151 and 75 bp
		CC	226 bp

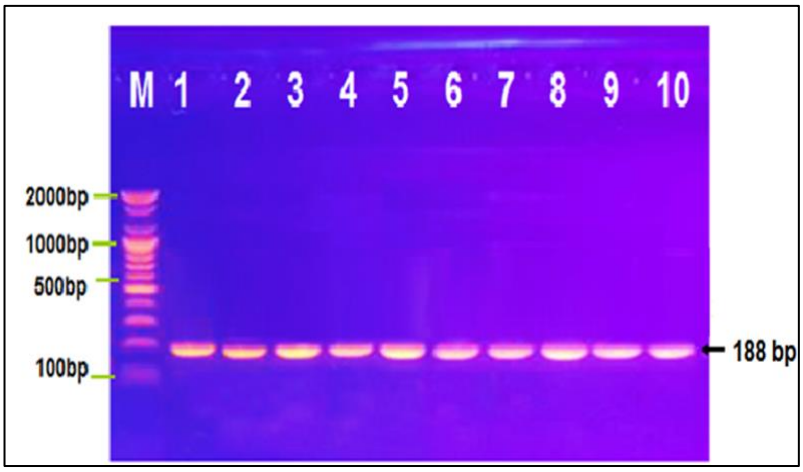

Figure 3.1 Agarose gel electrophoresis image that show the PCR product analysis of (TCF7L2 SNPs rs7903146) gene. From some blood patient samples (lane 1 – 5) and healthy control sample (lane 5 – 10)

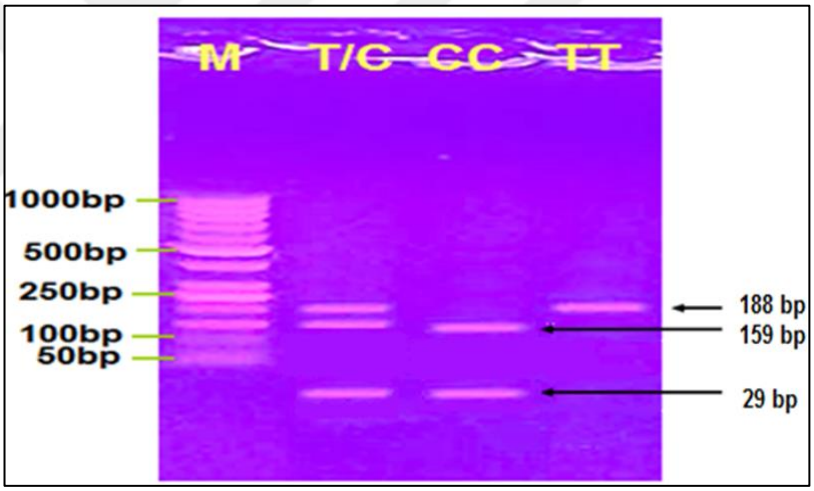

Figure 3.2 Agarose gel electrophoresis image that show the RELP-PCR product analysis of (TCF7L2 SNPs rs7903146) by using 2U RasI.

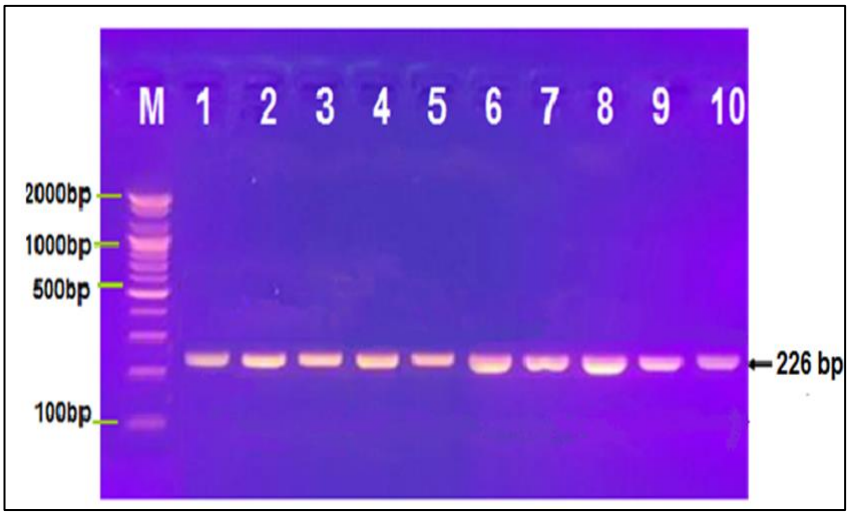

Figure 3.3 An picture of an agarose gel electrophoresis showing the PCR product analysis of the TCF7L2 SNPs rs12255372 gene. From certain blood patient samples (lane 1-5) and healthy control sample (lane 5 – 10)

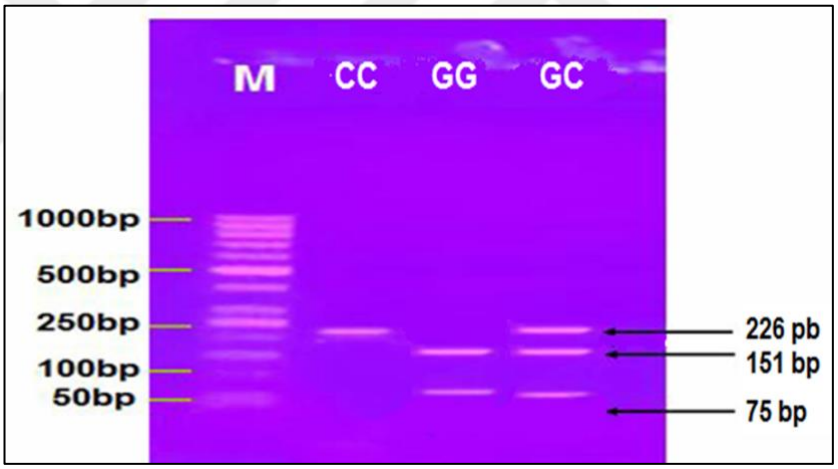

Figure 3.4 Agarose gel electrophoresis image, that show the RELP-PCR product analysis of (TCF7L2 SNPs rs12255372) by using Tsp 509I

3.3 Statistics Analysis

The information was then entered into a computerized database format. The statistical analysis was carried out with the assistance of a computer using SSPS version 23.

4. RESULTS AND DISCUSSION

4.1 Demographic Distribution of Sbject

In this study the number of diabetic patients are 100 and 100 persons as control groups, as shown in Figure 4.1.

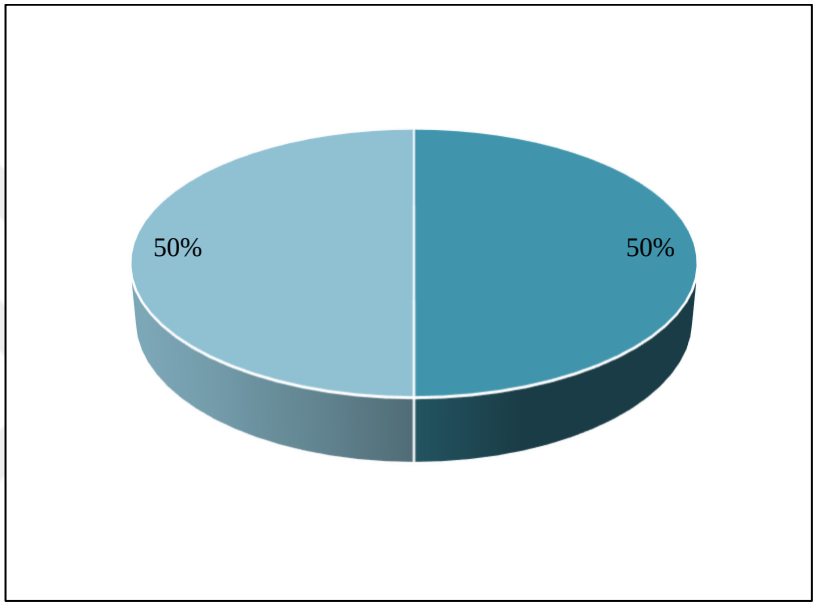

Figure 4.1 Distribution of DM patients according to gender

This research comprised a total of two hundred people who were representative of the overall population in Iraq. The exams carried out in the clinic and the laboratory determined that none of the one hundred people who made up the control group suffered from type 2 diabetes or any of the diseases that are often linked with it. The patient group comprised of one hundred people who had been diagnosed with T2DM according to the criteria established by the (WHO) (levels of fasting plasma glucose that were more than 126 mg/dL, or random plasma glucose that was greater than 200 mg/dL). Analyses were performed on each participant's laboratory data (triglyceride levels, fasting glucose, total cholesterol, high-density lipoprotein cholesterol, and HbA1c,), as well as their BMI, to determine their degree of obesity (Table 4.1). Individuals in both the sick group and the control group had to sign informed permission papers before they could take part in the

research. Additionally, the procedures had to get approval from the Ministry of Iraqi Health Ethics Committee in Research.

Table 4.1 An research of the clinical and metabolic features that are shared by diabetes patients and a healthy control group, as well as those that distinguish them from one another

PARAMETARS	**MEAN ± SD**		**P-VALUE**
	DM (N=100)	Control (N=100)	
Age (year)	55.12±9.65	54.74±8.93	>0.0.5
Duration of diabetes (years)	8.98±6.23	0	**<**0.05
Systolic BP (mmHg)	129.78±14.8	105.04±9.21	<0.05
Diastolic BP (mmHg)	80.63±21	69.61±4.02	<0.05
Fasting plasma glucose (mg/dl)	118.91±31.02	81.47±12.83	<0.05
Postprandial plasma glucose (mg/dl)	175.98±30.45	120.83±5.93	<0.05
HbA1c (%)	7.02±2.01	4.9±0.31	<0.05
Total cholesterol (mg/dl)	210.97±25.87	160.32±0.92	<0.05
Triglyceride (mg/dl)	177.98±22.94	122.61±90.82	<0.05
HDL-cholesterol (mg/dl)	42.69±11.09	57.92±11.41	<0.05
LDL-cholesterol (mg/dl)	141.62±23.55	101.83±20.8	<0.05
Serum creatinine (mg/dl)	0.89±0.12	0.5±0.2	<0.05
Serum urea (mg/dl)	44.87±5.92	22.81±5.91	<0.05
BMI	27.21±4.73	25.43±3.85	0.954
Microalbuminuria (mg/day)	200.63±5.88	11.04±2.81	<0.05

- The data are shown as the means together with the standard deviations. BMI stands for body mass index, while HbA1c stands for glycated hemoglobin.
- Statistically significant in chi-square test accepted If P<0.05.
- N = Number of Persons

Both of the (rs12255372) and (TCF7L2 SNPs, rs7903146), were successfully genotyped with a success rate of 98.55 and 97.42 percent, respectively. Both SNPs exhibited Hardy-Weinberg equilibrium in terms of the frequencies of their alleles and genotypes.

There was no discernible difference between the patients and the controls with regard to the genotype or allele frequencies for (TCF7L2 SNP rs7903146 allele). In the healthy control, the frequency of the (rs7903146) T allele was 28 percent, whereas in the sick

population, it was only 29 percent. The rs7903146 C allele frequency for the controls was 72 %, whereas it was 71% in the patients (P = 0.61). The frequencies of the T/T, C/C, and C/T genotypes were 54, 36, and 10 percent, respectively, for the controls, while the rates were 49, 44, and 7 percent, straight, for the patients (P = 0.30). (Table 4.2).

Table 4.2 Genotype and allele frequencies of the (TCF7L2 SNPs rs7903146) gene polymorphism in with diabetic patients and healthy control group

TCF7L2 SNPs rs7903146	**PATIENTS (N=100)**	**HEALTHY (N=100)**	**P.VALUE**
Genotypes	-	-	-
CC	49(49%)	54(54%)	0.32
CT	44(44%)	36(36%)	
TT	7(7%)	10(10%)	
Alleles	-	-	-
C	142(71%)	144(72	0.61
T	58(29%)	56(28%)	
P = <0.05, N= Number of persons			

In terms of genotypic and allelic frequencies for the TCF7L2 SNP rs12255372, there was no significant difference between patients and controls. The frequency of the rs12255372 T allele was 21 percent in the control group, but it was 27 percent in the sick group (P = 0.42). The frequency of the rs12255372 G allele was 79 percent in the normal group, and it was 73 percent in the patient group (P = 0.51). The T/T, G/G, and G/T genotype frequencies were, respectively, 63, 32, and 5% for the controls. However, they were, respectively, 52, 42, and 6% for the patients (P = 0.31); this indicates that the T/T, G/T, and G/G genotype frequencies were significantly lower in the patients. (Table 4.3).

Table 4.3 Genotype and allele frequencies of the(TCF7L2 SNPs Rs 12255372 polymorphism in with diabetic patients and healthy control group

TCF7L2 SNPs Rs 12255372 gene	**PATIENTS (N=100)**	**HEALTHY (N=100)**	**P.VALUE**
Genotypes	-	-	-
GG	52 (52%)	63 (63%)	0.31
GT	42 (42%)	32 (32%)	0.31

TT	6 (6%)	5 (5%)	0.31
+Alleles	-	-	-
G	146 (73%)	158 (79%)	0.51
T	54 (27%)	42 (21%)	0.42
P = <0.05, N= Number of persons			

Because of the difficulties that may arise from having T2DM, it has become a public health concern on a global scale. T2D is becoming an epidemic in a number of nations. For this reason, investigations on the complaint are becoming less relevant for the establishment of an early opinion strategy, successful population messaging strategies, more efficient treatments, as well as significantly enhanced prophylactic measures. recent spectacular growth in the value of diabetic cases exponentially shows that aspects connected to life, the expanding urbanization of metropolises, and a sedentary living in ultramodern mortal populations may play parts in driving the inheritable characteristics associated with (T2DM). The recent spectacular growth in the number of diabetes patients explosively shows that elements connected to life, the expanding urbanization of metropolises, and a sedentary existence in ultramodern deadly populations are contributing reasons. Therefore, in order to swiftly implement preventive measures, its essential to have a solid understanding of the inheritable features that are at the root of this complicated diseas (Ramachandran *et al*. 2002).

Based on the findings of both familial and binary research, the role of hereditary variables in the pathogenesis of type 2 diabetes has been extensively established (Groop *et al*. 1996, Poulsen *et al*. 1999). Nevertheless, it has been shown that the genes involved in T2DM have only a moderate effect on the risk of developing the disease, and studies that seek to reproduce the findings typically produce results that are inconsistent (Hattersley and McCarthy, 2005). According to results of a handful of research on the link between genetic variation and health outcomes, including the ones that evaluated (SNPs) in the CAPN10 genes, KCNJ11 Glu23Lys, and PPARG Pro12Ala, have been successfully repeated across a variety of different ethnic groups (Altshuler *et al*. 2000, Weedon *et al*. 2003, Gloyn *et al*. 2003). SNPs in the area containing the TCF7L2 gene have been increasingly related with T2DM in recent years (Grant *et al*. 2006, Cauchi *et al*. 2006).

In this thesis, we analyzed the link between T2DM and the rs12255372 as well as TCF7L2 SNPs rs7903146 in a sample from Iraq. Both the genotype and the allele frequency in the (rs12255372) and (TCF7L2 SNPs rs7903146) were comparable in the case and controls groups (P>0.05). On top of that, the allele frequency for these variants was not substantially connected with an increased risk of developing T2DM. Based on these findings, it is likely that the TCF7L2 SNPs rs7903146 and rs12255372 do not play a substantial role in the susceptibility of this group to develop type 2 diabetes.

In the beginning, it was thought that differences in (TCF7L2) gene were a significant factor in the development of type 2 diabetes in European populations in general (Groves *et al*. 2006, van Vliet-Ostaptchouk *et al*. 2007, Mayans *et al*. 2007, Scott *et al*. 2006). Despite this, recent research conducted on populations of a variety of racial and ethnic backgrounds, including Native American communities in North America and Chinese, has produced conflicting findings (Ren *et al*. 2008, Guo *et al*. 2007). These observations were corroborated by the observation that there was no connection between type 2 diabetes and (TCF7L2 SNP rs7903146), as well as that there was a borderline link between (rs12255372) and type 2 diabetes in a group from the UAE (Saadi *et al*. 2008). A further finding was that neither SNP was associated with an increased risk of diabetes in Saudi Arabians (Alsmadi *et al*. 2008).

It has been observed that (rs7903146) T allele in the (TCF7L2) gene is associated with a (1.57) times greater risk for type two diabetes, in a cohort of individuals who suffer from coronary heart disease. Iraq has one of the most diverse populations in the world. Within a diabetic population that was originally from the country of Brazil's central western region, Barra et al. found confirmation of these findings. Regarding the TCF7L2 SNP known as rs12255372, the research conducted by Furgeri and colleagues did not uncover any evidence linking this variation to an elevated risk of type two diabetes in cystic fibrosis patients. In the same vein, Franco et al. did a research that consisted of patients from the same area, and they found that the rs7903146 and TCF7L2 SNPs rs12255372 had no connection with T2DM in a Japanese-Brazilian groups. The results that we obtained from a community residing in northern Brazil complement the results (Franco *et al*. 2011, Furgeri *et al*. 2012), as well as they contribute to the assumption, that the

replication of the findings, in mixed populations doesn't really follow a same pattern, as that seen in more homogeneous populations, this is one of the reasons why the conclusion was reached. This was made clear by the fact that the pattern that our results followed did not correspond to the one that was seen in the population of Europe.

In a Saudi Arabian population, established a modest connection between the (rs12255372) and (TCF7L2 SNPs rs7903146) (D' = 0.80, R2 = 0.66) (Alsmadi *et al.* 2008). This was in reference to haplotype frequency. In addition, the researchers found a statistically significant difference (P = 0.03) in the CT haplotype between those diagnosed with type two diabetes as well as healthy controls. Despite this, the authors urge readers to take these findings with care because of the limited statistical power for this discovery. This is due to the fact that the CT haplotype was only identified in 38 of the 522 cases that were examined. Similarly, the findings of our research showed that there was a moderate correlation between the (rs12255372) and (TCF7L2 SNPs rs7903146) (D' = 0.65, R2 = 0.36) and that there was a tendency of an association between the CT haplotype and the population that was analyzed (P = 0.07). However, after accounting for multiple tests conducted on 1000 random permutations, the significance of this link was dramatically reduced (P = 0.18).

In spite of the fact that the SNPs in the TCF7L2 gene, have been shown to have a substantial connection with type two diabetes in a variety of studies, the mechanism by which the intronic mutations increase one's risk of developing the condition is still unknown. According to the findings of several studies, the gene TCF7L2 may have a significant role in the process of preserving the bulk and function of pancreatic (beta cells). This gene's product, known as a recap factor, may have an important function in, insulin storage, and protection against apoptosis (Shu *et al.* 2008, Loder *et al.* 2008). performed comprehensive functional research on (SNP rs7903146) in (TCF7L2 gene), and showed that this variation influences susceptibility to type 2 diabetes by affecting the cis-regulation as well as original chromatin structure in pancreatic islets cells (Gaultn *et al.* 2010). The findings of this study were published in the journal Nature Genetics. Identified an association between (beta cell) proliferation and the pancreatic expression of the (TCF7L2 gene) and the rejuvenescence of comparable cells in many different

animal models of diabetes (Shu *et al*. 2012). These findings were published in the journal Diabetes. On top of that, The research showed that when (TCF7L2 gene) was overexpressed in mortal exocrine pancreatic tissue, ductal epithelial cells began producing beta cell morphologies and insulin. This was discovered by the researchers.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

1. This is the first study to investigate at the TCF7L2 SNPs rs7903146 and rs12255372 in Type 2 Diabetes patients from a racially mixed community in Iraq. The results that are given here are just incomplete. Despite the fact that allele frequencies and the genotype in the patient groups as well as the control group, were equal ($P > 0.05$) for each variants, the patient group had a significantly higher frequency of both variations, Given the limited number of people that participated in the study, we cannot say for certain that the results we obtained are accurate, because we cannot rule out the possibility that our findings are skewed due to the small sample size. Alternately, such results might be ascribed to unique ethnic effects due to the fact that the majority of the previously reported connections were established in research with populations that were mostly European.
2. Although the TCF7L2 SNPs rs7903146 and rs12255372 do not exhibit a substantial connection, the modest association cannot be disregarded.

5.2 Recommendation

1. More study is required before reaching a judgment on the significance of these gene polymorphisms in diabetes type 2 among groups with a mix of different types of people.
2. Larger populations from places with more ethnic variability are needed to replicate the technique used in this study.

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