A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

ASSESSMENT OF ATP-BINDING CASSETTE TRANSPORTER G5/G8 AND LIPID PROFILE IN PATIENT WITH DIABETIC MELLUTIS TYPE 2

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

AMMAR ALI HUSSEIN HWESHIM

ÇANKIRI

2022

ASSESMENT OF ATP-BINDING CASSETTE TRANSPORTER G5/G8 AND LIPID PROFILE IN PATEINT WITH DIBETIC MELLUTIS TYPE 2

By Ammar Ali Hussein HWESHIM

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

Advisor **:** Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor **:** Asst. Prof. Dr. Antesar Rheem OBEAD

Examining Committee Members:

Chairman **:** Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Beytullah EREN
Environmental Engineering
Sakarya University

Member **:** Prof. Dr. Volkan EYUPOGLU
Chemistry
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Ammar Ali Hussein HWESHIM

ABSTRACT

ASSESSMENT OF ATP-BINDING CASSETTE TRANSPORTER G5/G8 AND LIPID PROFILE IN PATIENT WITH DIABETIC MELLUTIS TYPE 2

Ammar Ali Hussein HWESHIM

Master of Science in Chemistry

Advisor: Prof. Dr. Volkan EYÜPOĞLU

Co-Advisor: Asst. Prof. Dr. Antesar Rheem OBEAD

May 2022

The present study intends to look into some of the reasons of diabetes by looking at polymorphisms in the ATP Binding Cassette(ABC) G5/G8 gene in Heet Hospital and the relationship between these polymorphisms and lipid and ABCG5/G8 levels Serum lipid profile levels were determined using colorimetric methods, while serum levels of both ABCG5/8 were determined using the ELISA technique. In addition, ABCG5 and ABCG8 gene polymorphisms Q604E (rs6720173) and D19H (rs11887534) They are mapped using a polymorphism in the length of the restriction reaction fragment and the polymeric chain reaction, respectively. The mean ± SD ages of the patients between (20-60) years. Fasting plasma levels of TC, TG, and LDL-C were significantly higher in the patient group than in the control group (P-value >0.05). Despite the fact that there was a statistically significant variation between groups in the distribution of ABCG 5/8 protein, no significant changes were observed (p 0.05). Finally, because of its high connection with the E allele and link with ABCG 5 protein levels, the polymorphism (Q604E) of the ABCG5 gene was designated a risk factor for DM illness.

2022, 41 pages

Keywords: Dm, Pcr –Replp, Lipid profile, Snp, Elise

ÖZET

DİYABETİK MELLİTUSLU HASTALARDA ATP BAĞLAYICI KASET TRANSPORTER G5/G8 VE LİPİD PROFİLİNİN DEĞERLENDİRİLMESİ TİP 2

Ammar Ali Hussein HWESHIM

Kimya, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU

Eş Danışman: Dr. Öğr. Üyesi Antesar Rheem OBEAD

Mayis 2022

Mevcut araştırma, Heet hastanesinde ATP Binding Cassette (ABC) G5/G8 geninin polimorfizmlerini inceleyerek DM'nin bazı nedenlerini araştırmayı ve bu polimorfizmlerin lipid ve ABCG5/G8 seviyeleri ile ilişkisini araştırmayı amaçlamaktadır. Lipid profil düzeyleri kolorimetrik yöntemlerle ölçülürken, hem ABCG5/8'in ölçüm serum düzeyleri ELISA tekniğiyle hem de Polimeraz Zincir Reaksiyonu kullanılarak sırasıyla ABCG5 ve ABCG8 gen polimorfizmleri Q604E (rs6720173), D19H (rs11887534) belirlendi. Restriksiyon Fragman Uzunluğu Polimorfizmi, bu vaka kontrol çalışmasına 50 DM hastalığı olan hasta ve diğer sağlıklı bireyler dahildir. Hastaların ortalama ± SD yaşları (20-60) yıl. Açlık plazma TC, TG ve LDL-C seviyeleri, hasta grubunda kontrol grubuna göre anlamlı derecede yüksekti (P-değeri >0.05). ABCG 5/8 protein dağılımında gruplar arasında istatistiksel olarak anlamlı bir farklılık olmasına rağmen, anlamlı bir değişiklik gözlenmedi (p 0.05). Sonuç olarak, ABCG5 geninin polimorfizmi (Q604E), E alleli ile anlamlı ilişkisi ve ABCG 5 protein seviyeleri ile ilişkisi nedeniyle DM hastalığı için bir risk faktörü olarak kabul edildi.

2022, 41 sayfa

Anahtar Kelimeler: Dm, Pcr –Replp, Lipid profili, Snp, Elise

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Prof. Dr. Volkan EYÜPOĞLU and Asst. Prof. Dr. Antesar Rheem OBEAD, for his patience, guidance and understanding.

Ammar Ali Hussein HWESHIM

Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
µg	Microgram
µL	Microliter
µmol	Micromole
dL	Deciliter
g	Gram
L	Liter
m^2	Square-meters
mg	Milligram
mL	Milliliters

LIST OF ABBREVIATIONS

ABC	ATP-binding-cassette
ABCG5	ATP-binding-cassette G5
ABCG8	ATP-binding-cassette G8
BMI	Body mass index
Ch	Cholesterol
DM	Dibetic mellutis
DNA	Deoxyribonucleic acid
DW	Distilled water
HCL	Hydrochloric acid
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphisms
TG	Triglyceride
VLDL	Very low-density lipoproteins

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Diabetes mellitus is a metabolic condition that affects the metabolism of glucose, lipids, and proteins. Persistent hyperglycemia (high blood glucose) caused by insufficient insulin secretion, irregularities in insulin action, or a combination of both inadequate secretion and improper insulin action is what it's called (a hormone produced by the pancreas). It's fast becoming one of the most frequent chronic non-contagious illnesses, putting people's health in jeopardy all around the globe (Chaudhury *et al*. 2017).

Diabetes affects almost all of the body's organs, including the heart, eyes, kidneys, and nervous system, resulting in substantial financial and emotional hardship. As a result, early detection of the condition is crucial.

Diabetes mellitus (DM), a syndrome rather than a single illness, is the leading cause of mortality and disability in the world. Patients, their loved ones, and society as a whole bear a significant emotional and financial burden as a result of this major public health issue. Diabetes affects around 450 million people globally, or 8.8 percent of individuals aged 20 to 79, according to the World Health Organization. Diabetes is predicted to be the sixth greatest cause of death by 2030, according to the World Health Organization. (WHO) (Suk *et al*. 2016).

Diabetes symptoms include polydipsia, polyuria, polyphagia, impaired eyesight, and delayed weight reduction. Ketoacidosis or a non-ketotic hyperosmolar state may develop in the most severe forms of diabetes mellitus, leading to coma and, in the event of delayed treatment, death. There is a possibility that diabetes, either type 1 or type 2, is to blame (Latif *et al*. 2018).

A wide range of pathogenic processes, from autoimmune death of pancreatic -cells, which produces insulin insufficiency, to abnormalities that induce insulin resistance, are involved in the development of diabetes. The abnormalities in glucose, lipid, and protein metabolism found in DM are caused by insulin's inadequate action on target

tissues. Insufficient insulin synthesis and/or tissue responses to insulin cause insulin action to be reduced at one or more points along the insulin action pathways.Diabetes can be a side effect of other disorders, such as pancreatitis or haemochromatosis, which cause a decline in insulin secretion. Some endocrine diseases, such as Insulin antagonism exists in acromegaly and Cushing's syndrome action caused by aberrant hormone production with opposing activity Several. Drugs have an antagonistic effect on glucose tolerance (Boutayeb *et al.* 2013).

Aim of Study:

1. This case control study included investigating from some of the causes of diabetic mellutis through study polymorphisms of ATP-Binding Cassette, Subfamily G, Member 5 and 8 genes implicated in the principal metabolic pathways such as transport of cholesterol, and its role in the development of diabetic mellutis.
2. Evaluate serum levels of the lipids and of ABC G5/G8 proteins among patients of diabetic mellutis in AL Anbar governorate.
3. Investigate from the association of polymorphisms of ABC G5/G8 genes with lipids levels and ABC G5/G8 protein levels, and study variation in susceptibility.

2. LITERATURE REVIEW

2.1 Prevalence of DM

Diabetes mellitus is on the rise all across the globe. Diabetes prevalence in the adult population worldwide was 8.5 percent in 2014, almost twice the rate (4.7 percent) in 1980, according to a WHO global report on diabetes issued on April 6, 2016. IDF's 2015 Diabetes Atlas estimates that 415 million people globally have diabetes, and that number is expected to reach 642 million people by 2040. According to the same estimate, roughly five million individuals died as a consequence of diabetes in 2015, with diabetes and its complications accounting for (5-20%) of total health spending on adults. Furthermore, diabetes prevalence has lately increased significantly in nations that were not previously linked with a high frequency. The Middle East, where around 10% of adults have diabetes, and Africa, where over 20 million people have diabetes, with forecasts that this figure may double by 2035, are two such regions in the globe with a high incidence of diabetes (Ting *et al*. 2016).

2.2 Diagnosis of Diabetes Mellitus

The individual's life is influenced by the diagnosis of DM. As a result, both the doctor and the person being evaluated must have complete faith in the diagnosis. This is easy in symptomatic persons, but in asymptomatic people, if an aberrant test is discovered, it must be confirmed by another.

Plasma glucose remains the foundation for diagnosis since there is no biomarker in the definition of diabetes. To diagnose diabetes, blood sugar levels must fall below a particular threshold, which has been linked to a significant increase in microvascular complications. The Expert Committee on Diagnosis and Classification of Diabetes Mellitus (ECDDM) classifies fasting plasma glucose levels of 100–125 mg/dL and impaired glucose tolerance (IGT) values of 140–199 mg/dL as impaired fasting plasma glucose (IFG) (Benjamin *et al*. 2017).

2.3 Classification of Diabetes

The pathogenic mechanism that leads to hyperglycemia is used to classify DM.

2.3.1 Type 1 diabetes mellitus

Once known as insulin-dependent diabetic mellitus, T1DM is now commonly known as type 1 diabetes. This is the phrase used to describe a condition in which insulin therapy is required because the patient is at risk of developing ketoacidosis. It is more common throughout childhood or adolescence. About 5% to 10% of all diabetes diagnoses are attributed to type 2 diabetes mellitus (TIDM) (Ozougwu *et al.* 2013).

T1DM occurs when the pancreas' beta cells are destroyed, resulting in a lack of insulin. Type 1 diabetes mellitus (TIDM) is characterized by the body's immune system attacking and killing insulin-producing beta cells. Although beta cell death can take years, the disease's signs and symptoms usually appear within a few months (Gomes *et al.* 2018).

2.3.2 Type 2 diabetes mellitus

In people with type 2 diabetes mellitus (T2DM), the body is unable to produce enough insulin and glucose homeostasis is compromised due to a combination of non-autoimmune factors and multiple gene inheritance. It seems that its pathophysiology is a complicated combination of genetic predisposition and environmental factors. Adults are more likely to develop T2DM, which accounts for 90 percent to 95 percent of all diabetes diagnoses. People suffer relative insulin deficiency at some point in their life and on a regular basis. Some individuals may not need insulin treatment to keep alive (Karalliedde and Gnudi 2016). At least two major pathological defects have been identified in patients with T2DM: one is a reduction in insulin's ability to act on peripheral tissue, known as IR, which is widely regarded as the primary fundamental pathologic process, and the other is -cell dysfunction, which is the pancreas' inability to

produce enough insulin to compensate for insulin resistance (Pham and Eggleston 2015).

2.3.3 Gestational diabetes

It is high blood sugar and was identified for the first time during pregnancy, as at some stage of pregnancy it will cause problems for the child and the mother (Buckley *et al.* 2012).

On an international scale, prevalence varies from 5% to 10%, with certain diagnostic criteria influencing this. Data shows that gestational diabetes is linked to a variety of mental illnesses, including postpartum depression (Wilson et al. 2020).

2.4 Etiology of Type 2 Diabetes Mellitus

Many variables contribute to the incidence of this medical ailment, including sex, age, diet and fat as well as lack of sleep, but lifestyle, hereditary factors, and other medical conditions are the most significant (WHO 2016)

2.5 Complications of Diabetes Mellitus

Diabetes patients are at risk for a variety of health issues as time passes. Acute (with a rapid start and reversible) or chronic (with a prolonged onset and reversible) difficulties are classified (taking years or decades to develop). Hypoglycemia, hyperglycemia, and diabetic ketoacidosis are all short-term effects of diabetes (DKA). Microvascular (nephropathy, neuropathy, and retinopathy) and macrovascular (atherosclerosis) problems are the most common chronic complications of diabetes. (Diagnosis and Classification of Diabetes Mellitus Expert Committee, 2004).

2.5.1 Acute complications

Non-ketotic hyperosmolar state (NKHS) is more common than diabetic ketoacidosis (DKA) as an immediate result of type 1 diabetes (T1DM). T2DM patients are more likely to have the NKHS (American Diabetes Association 2014a).

Hyperglycemia, ketonaemia, and acidosis are all symptoms of DKA, which is a metabolic condition. A relative or total absence of insulin, as well as a surge in counter-regulatory hormones like glucagon and cortisol, produce DKA.Glycogenolysis and gluconeogenesis are accelerated as a result of hormonal instability, resulting in acute hyperglycemia. Increased lipolysis produces more free fatty acids (FFAs) in the blood, which are then metabolized during the ketogenesis process. This is accompanied by metabolic acidosis as a result of the aggregation of high numbers of ketone bodies. Acetoacetate, 3-beta-hydroxybutyrate and acetone are examples of ketone bodies. 3-betahydroxybutyrate is the most common ketone in DKA (Schwartz *et al.* 2016).

Accidental overadministration of insulin, meglitinides, or sulphonylureas is the most common cause of hypoglycemia. A large amount of insulin or a hypoglycemic medicine can precipitate hypoglycemia, on the other hand, the patient may have skipped a meal or done rigorous activity after receiving the typical dose of insulin or oral hypoglycemic drugs (American Diabetes Association 2014b).

2.5.2 Chronic complications of diabetes mellitus

DM is always linked to an elevated risk of microvascular disease (disease of the small blood vessels) and macrovascular disease (disease of the arteries).

Damage to blood vessels is the underlying cause of microangiopathy, which manifests as one or more of the following symptoms: retinopathy and nephropathy, as well as neuropathy (Hadjadj *et al.* 2016).

2.6 Diabetic Retinopathy

This condition, known as diabetic retinopathy (DR), affects both the peripheral and the central retina and may lead to blindness in diabetics as the illness advances. With a long time of DM (Joseph *et al.* 2016).

2.6.1 Diabetic neuropathy

Neuropathy, which can be mono-neuropathy, polyneuropathy, or autonomic neuropathy, affects about half of all persons with DM. Mono-neuropathy is a type of polyneuropathy that involves the malfunctioning of a single cranial or peripheral nerve. It is difficult to cure ulcers in people with diabetes because of the loss of peripheral sensation, which is compounded by a damaged microvascular and macrovascular junction in the periphery. Diabetic complications, such as diabetic autonomic neuropathy, are rather prevalent and may have life-threatening consequences. Temperature, blood pressure, digestion, bladder function, control, and even sexual function can all be affected by this type of peripheral neuropathy (Basher 2017).

2.6.2 Diabetic nephropathy

When it comes to chronic kidney failure, diabetes-related complications such as DN (diabetic nephropathy) are the most common cause. Renal failure is the major cause of ESRD because of excess extracellular matrix development in kidney mesangium and interstitial tissue (DN) (Selby and Taal 2020).

After kidney damage has started, it might take 5–10 years for signs of renal failure to appear. Itchy skin, headaches, extreme exhaustion, an overall sense of malaise, nausea, vomiting, a loss of appetite, and leg edema are just a few of the signs and symptoms (Hassan 2018).

2.7 ATP-Binding Cassette (ABC) Transporter.

There are several ABC transporters in the human and bacterial cell membranes that break down ATP to transport a broad range of substrates, including metal ions, peptides, sugar, cholesterol, plant hormones, and bile acids. The energy generated by ATP hydrolysis is converted into substrate trans-bilayer movement, either into or out of the cytoplasm (import and export) (Dean *et al*. 2001).

ABCs (also known as nucleotide-binding domains, or NBDs) in the cytoplasm and TMDs (also known as transmembrane domains) in the plasma membrane are involved in ATP hydrolysis and substrate translocation in both situations. There is an ATP-binding location of around 100 amino acids in the ABC transporters of most mammals where the dodecapeptide sequence known as Linker region (C region) connects the Walker A and Walker B motifs, as indicated in the previous paragraph (Figure 2.1, 2.2). 'Nucleotide binding domains,' also known as Walker motifs, are highly conserved (NBDs).

TM domains (TM) have six TM helices, which vary greatly across ABC transporters. This kind of transporter has just one ATP binding site and a single transmembrane domain. Two TMs and two NBDs make up functional transporters, on the other hand. A homodimer (an assembly of identical half-transporters) or a heterodimer may be formed as a result of ABC half-transporters acting together (assembly of diverse half-transporters). Based on structural and homological similarities, human DNA includes ABC transporters. Subfamilies are divided down into seven distinct groups (A-G) (Mishra *et al*. 2014).

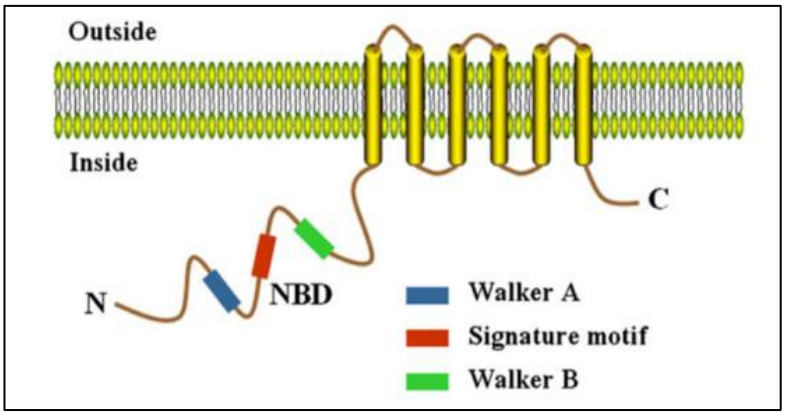

Figure 2.1 The structure of ABCG5 and ABCG8 proteins is shown in this diagram (Yu *et al.* 2010)

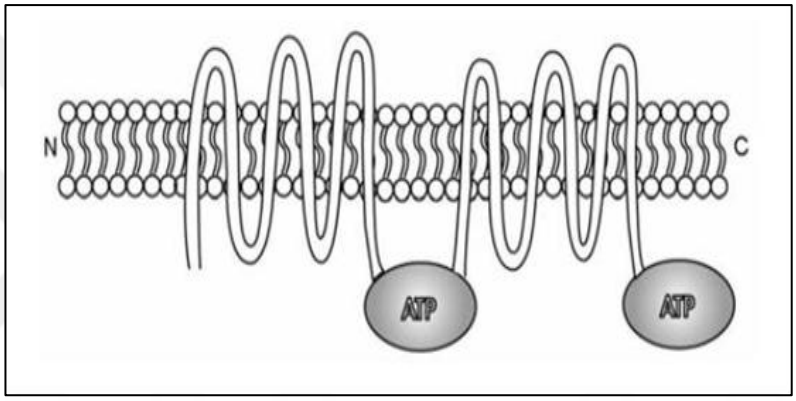

Figure 2.2 A complete ABC transporter is defined as a polypeptide including two transmembrane domains and two nucleotide binding domains (Tefková *et al*. 2004)

2.7.1 The ABCG-family

Homodimers and heterodimers of the family ABCG, such as ABCG5 and ABCG8, form functionally active homodimers or heterodimers by interacting with one another.

The NBD and C-terminal transmembrane domains are both found in ABC proteins (TMD). The five members of the human ABCG subfamily are ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. All three of these substances are produced by ABCG1 in cells: HDL, sphingomyelin, and phosphatidylecholine. In cancer cells, ABCG2 transforms xenobiotics and increases resistance to many anticancer drugs. RXR and

LXR, which are both adjacent to ABCG1, both activate ABCG4 and increase cholesterol efflux to HDL, hence enhancing the efficacy of statins. The apical liver and the small intestine membranes express ABCG5 and ABCG8 (Vasiliou *et al*. 2009).

2.7.2 The ABCG5/ ABCG8 transporter

G5 is an ATP-binding cassette, a protein that binds to ATP. The ABC system's two half-transporters (ABCG5 and ATP-binding cassette G8) may each work independently of the other's functions. Hepatobiliary, gallbladder, and intestinal cancers predominate. A brief intergenic region separates the Abcg5 and Abcg8 genes on chromosome 2p21, where they are found in a head-to-head arrangement (Figure 2.3). This common inter-genic promoter contains response elements for a variety of transcription factors, including liver X receptors (LXR), hepatocyte nuclear receptor 4 (HNF4), orphan nuclear receptor liver receptorhomolog-1 (LRH-1), forkhead box protein O1 (FOXO1), and thyroid hormone receptor. It also guarantees that both G5 and G8 are expressed at the same time, which is essential for the formation of the heterodimeric protein complex and cell surface trafficking. Humans have a 374-bp bidirectional shared promoter region that ensures coordinated gene regulation (Khunweeraphong *et al*. 2020).

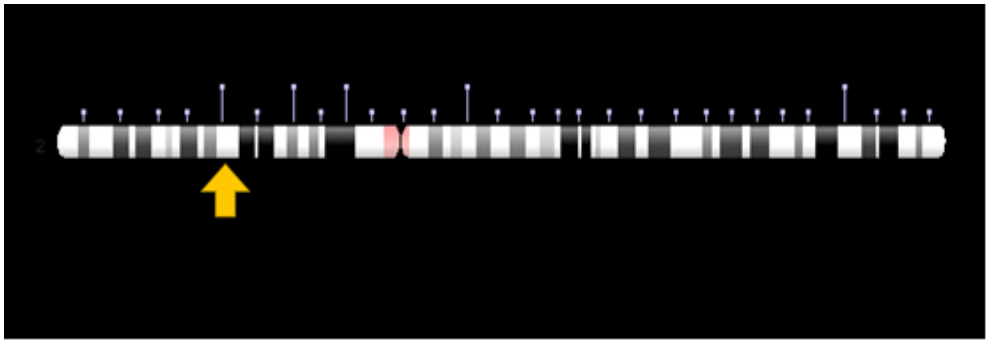

Figure 2.3 Chromosomal location of ABCG5/G8 (Hazard and Patel 2007)

Endoplasmic reticulum glycoproteins ABCG5 and ABCG8 have previously been identified (ER). N-linked glycan-dependent heterodimerization of G5G8 promotes correct protein folding in the ER. Connections to both half-transporters are made possible by calnexins, lectin chaperones, and the calreticulin protein. After forming in

the Golgi apparatus in the liver and small intestine, it is mostly expressed on the apical surface of hepatocytes (Figure 2.4).

G5G8 is the most major hepatobiliary elimination mediator, accounting for 70% to 90% of biliary cholesterol secretion. Bile salt micelles are essential to effectively mediate cholesterol efflux. G5G8 also blocks phytosterol absorption in the small intestine.

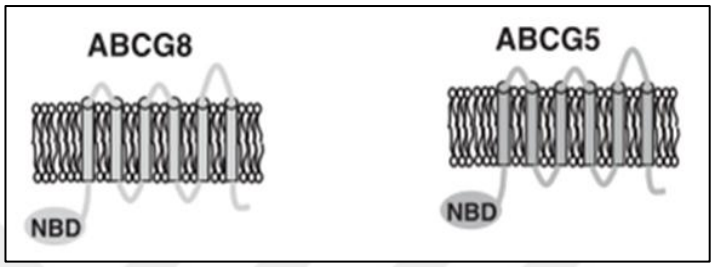

Figure 2.4 ABCG5 and ABCG8 ATP-binding cassette G8 are represented schematically in ABCG5, or ATP-binding cassette G5, is a protein that binds to ATP. ABCG8, or ATP-binding cassette G8, is a protein that binds to ATP (Zhang *et al.* 2016)

2.7.3 ABCG5/G8 genotype and plasma cholesterol

Cholesterol is a component of cell membranes that is essential for the operation and proliferation of all mammalian cells. It can be synthesized from acetyl-CoA or obtained through the diet. People who eat Western diets have 1 g of cholesterol per day, with 50% of that being absorbed. Through biliary secretion, an identical amount of cholesterol is removed from the body, either as free cholesterol or following bile acid conversion.

Hepatocyte apical membranes include ATP-binding cassette (ABC) proteins ABCG5 and ABCG8, which form heterodimers and help in the synthesis of biliary cholesterol (G8). People missing G5 and G8 (G5G8) have dramatically decreased biliary cholesterol release, and their hepatic and plasma cholesterol levels are very vulnerable to dietary cholesterol fluctuations. Transgenic mice overexpressing human G5 and G8 enhanced biliary cholesterol release, but not biliary cholesterol concentrations. G5G8

expression must be increased to increase biliary cholesterol release, according to this research (Zein *et al*. 2019).

The ABCG5 and G8 genes have gotten a lot of attention for their role in plasma lipid homeostasis. Specialized testing found that those with the ABCG5 Q604E mutant allele had higher baseline plasma total cholesterol (TC) levels than those with the wild-type gene's allele. Genotype showed no influence on plasma TC and low-density lipoprotein cholesterol effects of low- and high-cholesterol diets (LDL-C). According to a recent research, plasma lipids and the ABCG5 and ABCG8 gene polymorphisms are connected. siblings of DM sufferers with gallstones had higher triglyceride and poor HDL cholesterol levels than DM sufferers without a family history of gallstones.

Low cholesterol absorption efficiency was strongly linked to the ABCG8 19H risk allele, as was a higher rate of cholesterol excretion in bile. There was also a reduction in total cholesterol and low-density lipoprotein cholesterol levels among those who had the risk alleles. The ABCG8 19H variant seems to decrease cholesterol absorption in the gut and serum cholesterol levels, but it also causes an increase in hepatic cholesterol synthesis, which has been related to cholesterol gallstone susceptibility. Hepatic cholesterol synthesis rose two to fourfold, but neutral sterol excretion in the feces increased three to six times. The size, content, or excretion of bile salts did not alter ((Zhang *et al*. 2020).

3. MATERIALS AND METHODS

3.1 Subjects

3.1.1 Study settings

During the months of December 2021 and January 2022, patients who visited a Debetic mellitus center and came to test their glucose levels at General Hospital in Heet and Ramadi Teaching Hospital in Anbar province were studied.

The study's practical component took place at the Department of Biochemistry's laboratory at Babel University's College of Medicine.

3.1.2 Study population

The study were distributed into two collections:

The first group comprises 50 symptomatic patient with type 2 diabetic mellitus for age 20-60 years, based on clinical presentation, while the second group includes 50 apparently healthy control for age 20–60 years.

Everyone was asked about their age, medical history, and BMI, which was determined using (BMI= weight (kg) / height m^2) as the formula.

3.2 Material

3.2.1 Chemicals

Table 3.1 provides a list of the substances and equipment used in this experiment.

Table 3.1 Chemical substances used in the study

N0	MATERIAL	COMPANY	ORIGIN
1	Agarose	Bio Basic	(China)
2	ATP-Binding Cassette 5 ELISA Kit	Bioassay technology labratory	(China)
3	ATP-Binding Cassette 8 ELISA Kit	Bioassay technology labratory	(China)
4	Cholesterol Kit	AGAPPE	(Switzerland)
5	Triglyceride kit	AGAPPE	(Switzerland)
6	HDL-C	AGAPPE	(Switzerland)
7	Ethanol	Biosolve company	(USA)
8	PCR Master mix	invitrogen	(USA)
9	Primer ABCG5	Amsbio	(USA)
10	Primer ABCG8	Amsbio	(USA)
11	Restriction enzymes TBE buffer	Thermofisher	(USA)
12	50 bp DNA ladder	BIORON Diagnostics GmbH	(Germany)
13	Safe Red	Chembio	(USA)
14	Genomic DNA extraction	Intron Biotechnology (Korea)	(Korea)

3.3 Instruments

Table 3.2 lists all of the items utilized and their origins in this investigation.

Table 3.2 Tools and instruments utilized in this investigation are shown

NO	INSTRUMENTED TOOLS	ORIGIN
1	Cooling Centrifuge	PrismR / USA
2	Deep Freeze	GFL / Germany
3	Disposable syringes	(5 mL)
4	Distiller	GFL / Germany
5	EDTA tube (5mL)	AFCO, Jordan
6	ELISA reader and washer	Biotech / USA
7	Eppendorf tube (1.5mL)	China
8	Hood	4labtech / Korea
9	Horizontal gel electrophoresis (agarose)	Autto / Japan
10	Hot plate	Grant / England
11	Gel tube (2.5mL)	AFCOVAC (Jordan)
12	Incubator	Fisher Scient. / Germany
13	Micropipettes (5 -50µL), (2- 20µL), (20-200µL), (100-1000µL)	Slamed / Germany
14	PCR Thermo cycler	Bioneer / Korea
15	Photo documentation	E – Graph / Japan
16	Spectrometer	Varian/Austria
17	Scan drop	Analytikjena / Germany
18	Sensitive	balance
19	Vortex (Electronic)	Cleaver/ UK
20	Water bath	GFL / Germany
21	1, 0.1, 0.01 mL pipette tips	China
22	Filter papers	China

3.4 Chemical and Kits

The Lot number of the kits used in this investigation is shown in Table 3.3.

Table 3.3 Chemical and Kits used

NO	KITS	LOT N.
1	ATP-Binding Cassette 5 ELISA Kit	MBS2033842
2	ATP-Binding Cassette 8 ELISA Kit	MBS2034340
3	Cholesterol Kit	30110511
4	Triglyceride kit	30110479
5	HDL_C	30090404
6	PCR Master mix	07BIXPA9125Q1ZF
7	Primer ABCG5	HP214452
8	Primer ABCG8	HP214453
9	Restriction enzymes TBE buffer	IVGN3004
10	50 bp DNA ladder	304008
11	Safe Red	21141
12	Genomic DNA extraction	17231

3.5 Methods

3.5.1 Determination human ATP binding cassette transporter G5 (ABCG5)

Principle: Enzyme-linked immunosorbent assays are included in this bundle (ELISA). The plate was precovered with an antibody from Human ABCG5. The well-covered antibodies bind to ABCG5 in the sample, which is attached to ABCG5 in the sample. The material is then incubated with biotinylated Human ABCG5 Antibody, which recognizes ABCG5. After incubation, the unbound Streptavidin-HRP is washed away during a washing step. Afterwards, a substrate solution is added, and the color develops in direct proportion to the amount of Human ABCG5. 450 nm of absorbance is measured after adding acidic stop solution to the process.

3.5.2 Determination human ATP binding cassette transporter G8 (ABCG8)

Principle: There is an ELISA test included in this package for your convenience (ELISA). Human ABCG8 antibody was used to coat the plate. This connects to the sample's ABCG8 and binds to the antibodies that have been well coated. Finally, biotinylated Human ABCG8 Antibody is introduced and identifies ABCG8 as the target of its binding. After incubation, the unbound Streptavidin-HRP is washed away during a washing step. In a proportion to the volume of Human ABCG8, a substrate solution is then added, and the color develops. 450 nm of absorbance is measured after adding acidic stop solution to the process.

3.5.3 Extraction of DNA sample

Principle : By using the iNtRON kit, it can be obtained a very fast, simple technique for extraction intact and purified DNA from human blood. Silica matrices incorporated in spin columns have special properties for DNA binding. They have a great affinity for the DNA's negative charge since they have a positive charge. The use of sodium cations that firmly attach to the negatively charged oxygen of the DNA phosphate results in high salt conditions and pH. DNA was extracted in an elution buffer after numerous washing processes to eliminate contaminants. acidic elution at pH 7 in a buffer of tries-EDTA. Isolated DNA will be characterized by high quality and suitable to use in convential PCR Four simple steps are used in this system.

1. Release of the DNA by disrupting or homogenizing the blood.
2. The DNA bind to spin column.
3. Eliminating impurity by washing solution.
4. The DNA that purify will be eluted

3.5.4 Estimation the DNA purity and concentration by using nano photometer

Absorbance measurement is the most frequent technique for estimating DNA yield and purity. The absorbance of DNA to light is measured at 260 nm, which is the wavelength at which it absorbs the most light. The ratio of the absorbance at 260nm divided by the reading at 280nm was estimated for the purpose of determining the purity of DNA. A 260/A280 ratio of 1.7- 2 indicates that the DNA is of high quality. The lower the ratio, the more pollutants there were.

Analyzing the turbidity of the A260 turbidity test, increasing the dilution factor by 50 (g/mL of pure DNA), and using an A260 of 1.00 = 50 (g/mL) as a starting point to compute DNA concentration -

50 g/mL Conc. (g/mL) = (A260 reading — A320 reading) * dilution factor * (A260 reading — A320 reading).

- The final volume of the purified entire sample was multiplied by the DNA concentration to get the total production.
- DNA yield (in g) = DNA concentration times the total volume of a sample (mL)
- Using a scan nano-drop technology, we were able to determine the purity of the DNA

3.5.5 Agarose gel electrophoresis

For separating proteins and DNA, agarose gel electrophoresis is a commonly used method. With the help of an electric current, nucleic acid molecules were sorted by size, and the negatively charged molecules moved to the positively charged anode.

The flow is exclusively determined by molecular weight, with smaller molecules moving faster than bigger molecules. Furthermore, agarose gel electrophoresis may be used to fractionate nucleic acids as a preliminary step in the purification of the desired

bands. After digesting DNA fragments with appropriate restriction enzymes, agarose gel electrophoresis is a widely used method for measuring the size of DNA fragments.

3.5.6 Loading of DNA and electrophoresis

5 l of DNA was extracted and combined with 2 l of DNA loading dye (bromophenol blue and xylene cyanol). The agarose gel was carefully loaded with the samples, and the electricity was turned on at 70 volts/cm2 for two hours. The DNA was then transported from the negative electrode (cathode) to the positive electrode (electrode) (anode). A UV transilluminator was used to see the DNA bands on the gel..

3.5.7 Polymerase chain reaction–restriction fragment length polymerase

The polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method was employed to genotype individuals. Restriction enzymes are enzymes that are able to recognize short and particular DNA sequences.

It based on amplification of segment genome in to millions of copies. it include denaturation at 94-95°C to entails the double strand DNA in to two single stands, annealing at 55-65°C to allow a pair of primers (forward and reverse) to anneal to end of the template and final step of extension that include extension of DNA synthesis starting from the primes using DNA polymerase (from bacteria : Thermus aquaticus), This step occurs at 72-74°C.

PCR-RFLP was used to identify a single nucleotide polymorphism gene. Amplification was achieved in a programmable thermal cycler gradient PCR system.

3.5.8 Primer

It is (17-26) base pare single strand oligonucleotide sequences that serves as a initiating start for formation of DNA. Polymerases enzyme use the 3'-end of these primers as a

start to add nucleotides each time to form a single DNA strand. A lyophilized form of the primer was delivered. Lyophilized primers were reported in picomoles per unit mass. Re-form the primer in sterile nuclease – free H2O – to generate a stock of primer. There is a quantity of H2O supplied by the manufacturer that may be added to each primer to create a master stock that can be reused for subsequent batches.

The primers were reconstituted and diluted in the following procedures:

1. The tube was steadily deflating until the top was popped off.
2. Following the manufacturer's instructions for making Master Stock, the correct quantity of water was used to achieve 100 ppm (parts per million).
3. Using the vortex tube, you may re-suspension primers evenly.
4. Eppendrof was used to transfer the master stock, which contained 90 l of sterile nuclease-free H2O, into a 0.2 mL tube (Working stock).
5. At -20Co, the bulk of the stock was held.
6. At -20 degrees Celsius, the work stock.
7. Stocks were defrosted and centrifuged until they could be used in the PCR.

Upon reconstitution of the primers and delivery into single-use aliquots. Creating single-use aliquots can restrict the freezing of primers, thereby extending their life. The primer sequence was used for ABCG 5/8 gene according, as show in the following Table 3.4, 3.5.

Table 3.4 Sequence of primer were used for PCR amplification of ABCG 5 gene

PRIMER	SEQUENCE	LENGTH
Forward	5’- CTTTCACTACCTGCTAATGAGATG-3’	24mer
Reverse	5’GAGATAAACCACACCTGACACTG- 3’	23mer

Table 3.5 Sequence of primer were used for PCR amplification of ABCG8 gene

PRIMER	SEQUENCE	LENGTH
Forward	5’ – TTATCTGATGTACCTTTAGCCAGCG – 3’	25mer
Reverse	5’ – ATGTCCTCACACTGCTTGATGTCC – 3’	25mer

3.6 Statistical Analysis

All statistical analyses were performed using SPSS ® 23.0 on Windows ®. The difference was deemed significant when the p-value was less than 0.05. Using the one-way ANOVA test, cholesterol levels may be compared between individuals with various genetic backgrounds.

4. RESULTS AND DISCUSSION

4.1 Study Subjects' Demographic and Biochemical Characteristics

The demographic and biochemical data of the 100 research participants were adjusted for matching criteria such as age, as shown in Table 4.1. Patients and controls had no statistically significant difference in age mean variance (P > 0.05). This matching is required to remove any disparities in findings that may occur due to age differences. The same matching concept is used to remove the influence of any confounding variables in the illness association. Furthermore, the BMI and study groups had a non-significant outcome (p > 0.05). Diabetes and healthy individuals had significantly different fasting total cholesterol (TC), triglyceride (TG), HDL (HDL), and LDL (LDL) cholesterol levels (P 0.05). (P 0.05). This shows a relationship between lipid levels and diabetes mellitus, as shown in Figure 4.1, 4.2, 4.3 and 4.4 (Kusuhara and Sugiyama 2007).

Table 4.1 The 100 study subjects' demographic and biochemical characteristics

NO	CHARACTERISTICS	MEAN ±SD		P-VALUE
		Control N = 50	Patient N = 50	
1	Age (year)	30.44 ±12.485	37.36 ± 9.720	P > 0.05
2	BMI (Kg/m2)	25.80 ±5.617	26.4780 ±4.465	P = 0.009
3	TG (mg/dL)	146.22±39.44	350.58 ±83.70	P > 0.05
4	TC (mg/dL)	149.72±42.96	330.68 ±36.38	P > 0.05
5	HDL-C (mg/dL)	38.10 ±8.40	74.30 ± 7.5	P > 0.05
6	LDL-C(mg/dL)	82.72 ±40.28	150.28 ± 53.3	P > 0.05

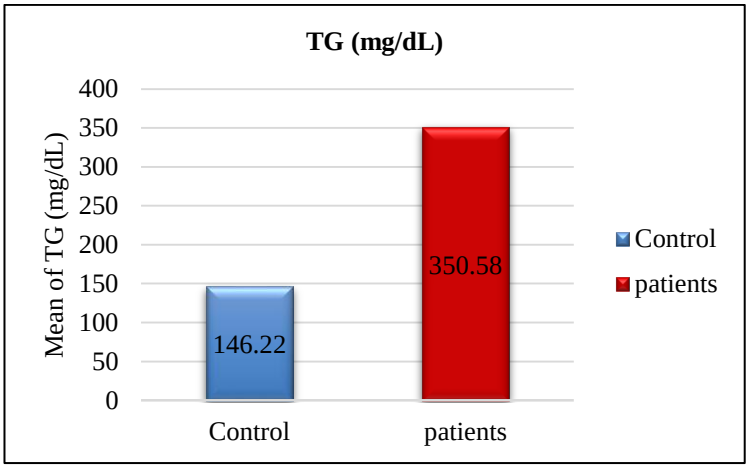

Figure 4.1 Show distribution of the mean of TG among study groups

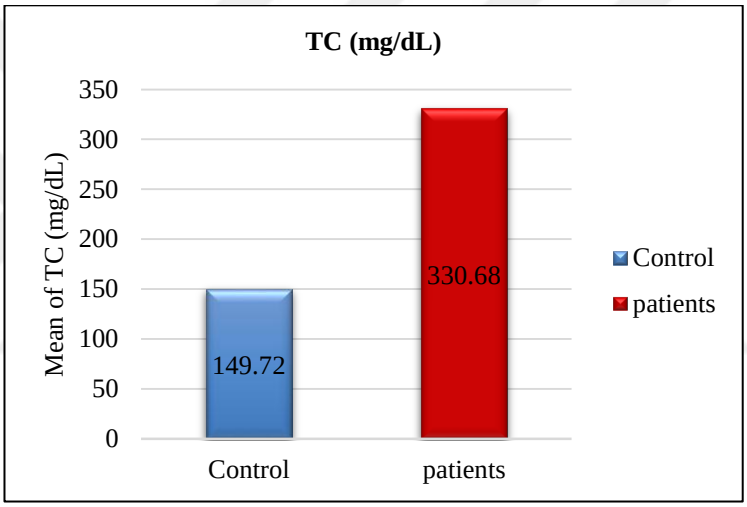

Figure 4.2 Show distribution of the mean of TC among study groups

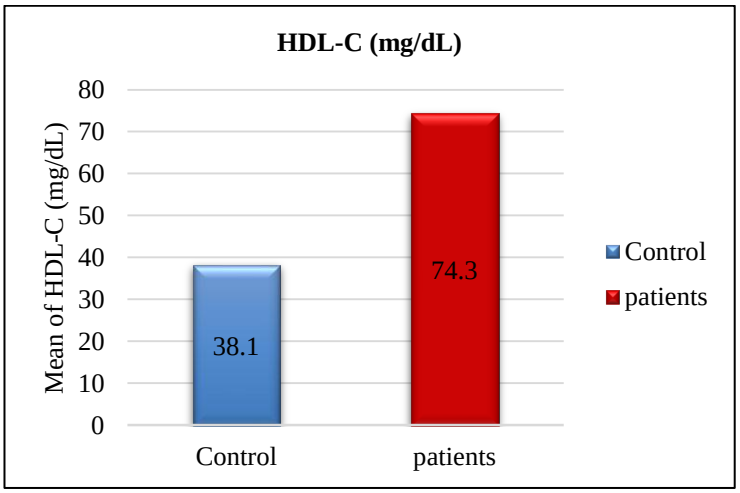

Figure 4.3 Show distribution of the mean of HDL among study groups

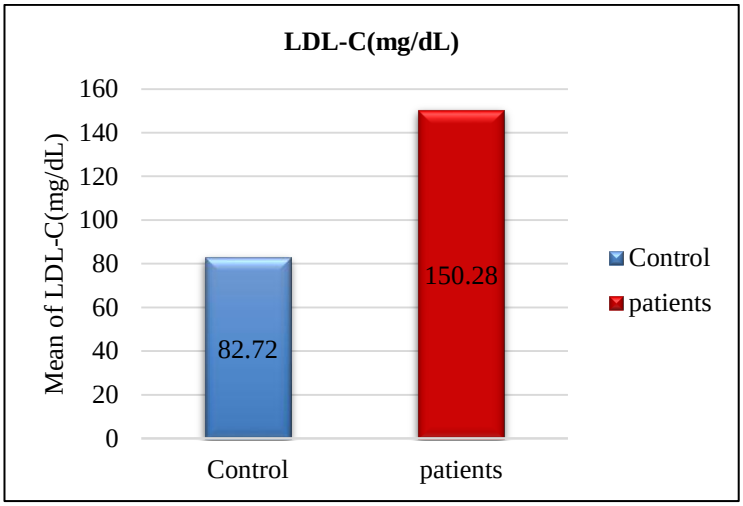

Figure 4.4 Show distribution of the mean of LDL among study groups

4.2 Age

In our study, the general mean age of patients with DM and control were (30.44 ±12.485) and (37.36± 9.720) years old, respectively, Because DM are rarely dissolved impulsively, the cumulative prevalence of DM increases with age, as shown in Figure 4.5 (Pham and Eggleston 2015)

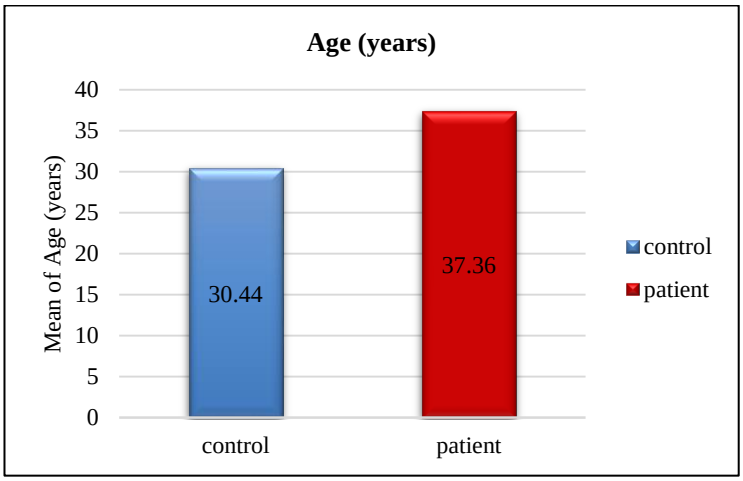

Figure 4.5 Show distribution of the mean of age among study groups

Where the peak age of patients with DM was between 20-40 years, This often agreement with other studies during which duration and elevated level are credited as causes for the age difference. Jindal et al displayed that, estrogen in turn increases

cholesterol level causing elevated of glucose in blood. Furthermore, it was shown that using more oral contraceptives and losing weight quickly increases the risk of gallstones in this age range. A major impact is also played by hereditary factors. Return reasons are growing in Iraq, despite a clear drop in presentation age, which might be attributed to traumatic life circumstances to which Iraqi individuals have been subjected (Daz et al. 2012).

Unlike other studies that have indicated an older age to be a major risk factor for diabetes, this study did not find an older age to be a significant risk factor. Long-term exposure to risk factors and sedentary behavior, which is more prevalent in the elderly than in the younger population.

4.3 Body Mass Index (BMI)

As shown in Figure 4.6, the mean body mass index (BMI) of the analyzed groups was 26.4780kg/m^2 in diabetics and 25.80 kg/m^2 in healthy controls, with no significant difference (p = 0.09). This investigation supported the findings of H. Thamrin et al, who found that BMI could not be used to predict the occurrence of DM complications in an individual. BMI and waist circumference, in contrast to Kiani et alresults, .'s were significantly linked to higher levels of DM (Riddy *et al.* 2018).

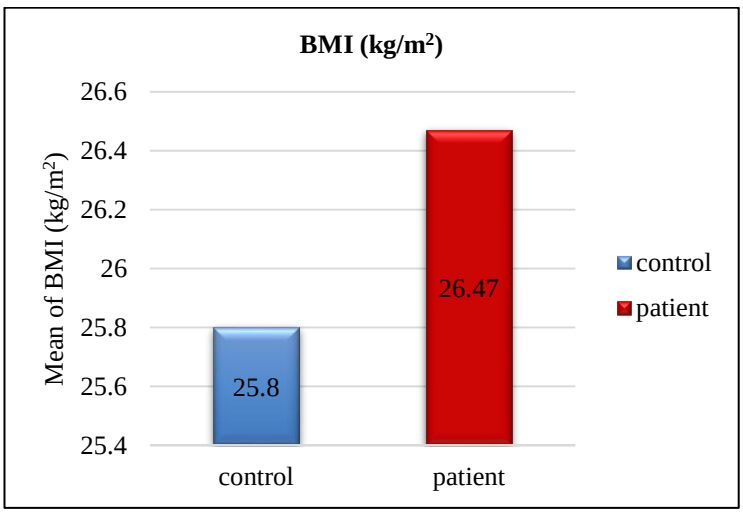

Figure 4.6 Show distribution of mean of BMI among study groups

4.4 Biochemical Characteristics Comparison of Patients with DM Disease and Control Groups

When comparing diabetics to healthy controls, the mean concentrations of Triglycerides (TC), LDL-C, and TG were significantly different. This indicates that even people with normal lipid parameters can develop DM. According to Weerakoon et al. the beneficial effects of female reproductive hormones on serum lipids can be considered as a possible rationale for such observations (Suk *et al.* 2016).

Although the association between high cholesterol and DM was statistically significant, the patient's mean blood cholesterol levels were greater than the control group's. According to the study, DM patients had significantly higher levels of blood cholesterol than the control group (S. Hayat *et al*. 2011). In their work, H. Weerakoon et colleagues discovered comparable findings. In contrast to previous results, J. Wang et al. observed that blood cholesterol levels in diabetic patients were significantly greater than those in the control group.

Triglyceride levels in patients were substantially higher than in the control group, according to this study. Some researches observed a significant relationship between serum triglycerides in DM patients and control groups, and this result matched their findings. M. Shadd came at the same conclusion. Furthermore, Hayat et al. demonstrated that, although some evidence shows that triglycerides in the intestine diminish DM motility, the research on the relationship between blood triglyceride levels and DM development is mixed (Schwartz *et al*. 2016).

The patients' mean blood HDL levels were lower than the control group in the present investigation, but the difference was statistically significant. Similar findings were found in research done by R. Singh et al. On the other hand, Alishi et al. discovered that low HDL cholesterol is linked to diabetes and that HDL cholesterol is the major source of cholesterol. There was a statistically significant difference between the LDL levels in the patients and those in the control group in this investigation. According to R, there was no difference between the experimental and control groups in terms of LDL

concentration. Singh et al. Other studies have identified a relationship between blood LDL levels and DM patients, but our data contradicted these (45) There have been studies that suggest a relationship between high LDL levels in the blood and diabetes, as well as those that show no link. However, in the present research, patients' blood LDL levels were higher than the control group's.

4.5 Elisa Measurement of Serum ATP Binding Cassette G5/ATP Binding Cassette G8 (ABCG5/58) Levels.

The blood levels of ABCG 5 and ABCG 8 varied substantially between the study groups (p= 0.0001) and the control group (p= 0.006), as shown in Table 4.2, with the patient group significantly lower than the control group.

Table 4.2 Serum ATP binding cassette G5/ ATP binding cassette G8 (ABC G5/G8) concentrations were determined in the study group

CHARACTERISTICS	CONTROL N=50		CASE N=50		P- VALUE
	Mean	SEM	mean	SEM	
ABCG5 (ng/mL)	2.30	0.37	0.60	0.12	0.0001**
ABCG8 (ng/L)	600.08	106.85	310.28	50.46	0.0013*

*P < 0.05, SEM = standard error of mean, N: number, ABCG5, Serum ATP binding cassette G5, ABCG8: Serum ATP binding cassette G8.

Plasma levels of ABCG proteins were considerably lower in the patient group as compared to the control group, as seen in Figures 4.7, 4.8, which might be related to alterations in protein secondary structure (Pham and Eggleston 2015). It may be due to changes in the form of the protein or the quantity of it produced, resulting in the lowest, or it could be due to changes in the shape of the protein or the amount of it produced, resulting in the lowest.

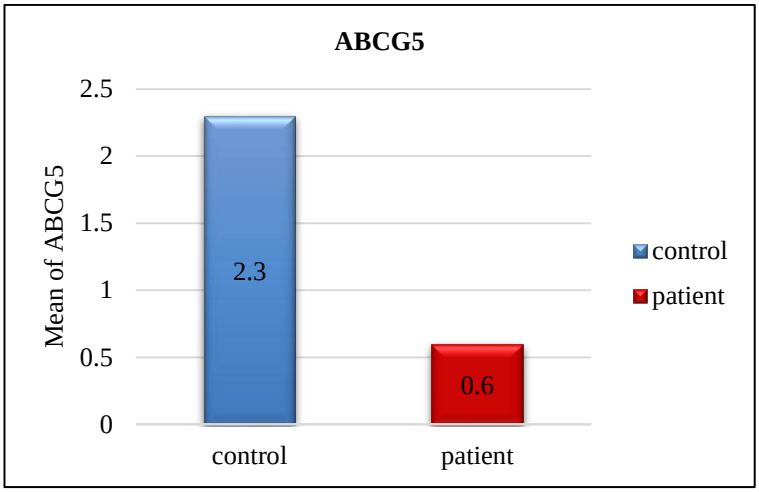

Figure 4.7 Determination mean of serum ABCG5 concentration in studied group, mean of ABCG 5 (ng/mL) concentration

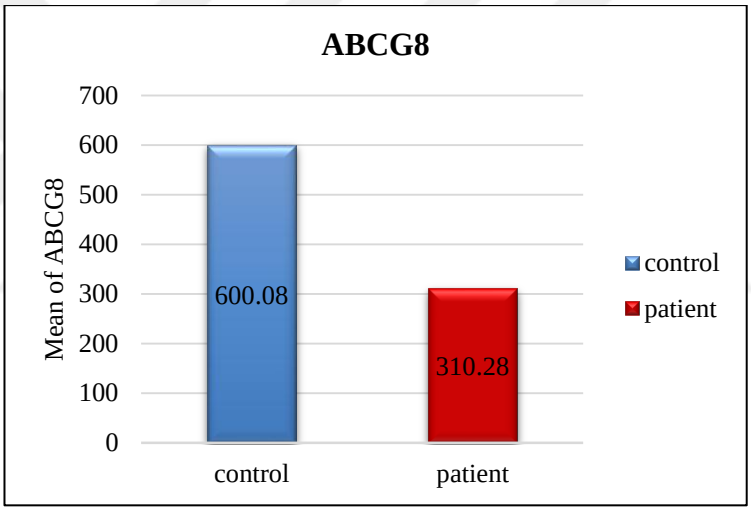

Figure 4.8 Determination mean of Serum ABCG8 concentration in studied group, mean of ABCG 8 (ng/L) concentration.

4.6 DNA Detection, Concentration and Purity

A. Electrophoresis of Extracted DNA on an Agarose Gel:

Figures 4.9 show DNA bands of some of the investigated samples, which were electrophoresed to ensure that there was a product and to give a notion of the precision of the extraction procedure.

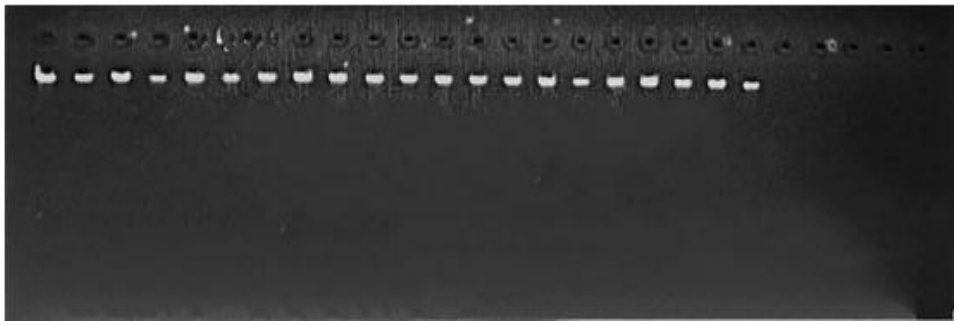

Figure 4.9 The extracted DNA electrophoresis on 1% agarose, 100V, and for 60 minute (5 µL of extracted DNA loaded in each well). extracted DNA.

B. DNA Concentration and Purity Measuring:

Concentrations of isolated DNA varied from 30 to 100 ng/µL and the purity (A260/A280) was between 1.8 and 2.

4.7 Detection of ATP Binding Cassette G5 (ABCG 5) Gene Polymorphism

4.7.1 Polymerase Chain Reaction Product

The present study's DNA samples were amplified using specified forward and reverse primers, yielding a 240 base pair band, which was our goal.

A. PCR Product ABM Master Mix:

Polymerase chain reaction (PCR) Product using abm Master Mix give sharp and apparent 240 bp band as Figure 4.10 shown, therefore this study choose it in the rest of the work because of its high reliability

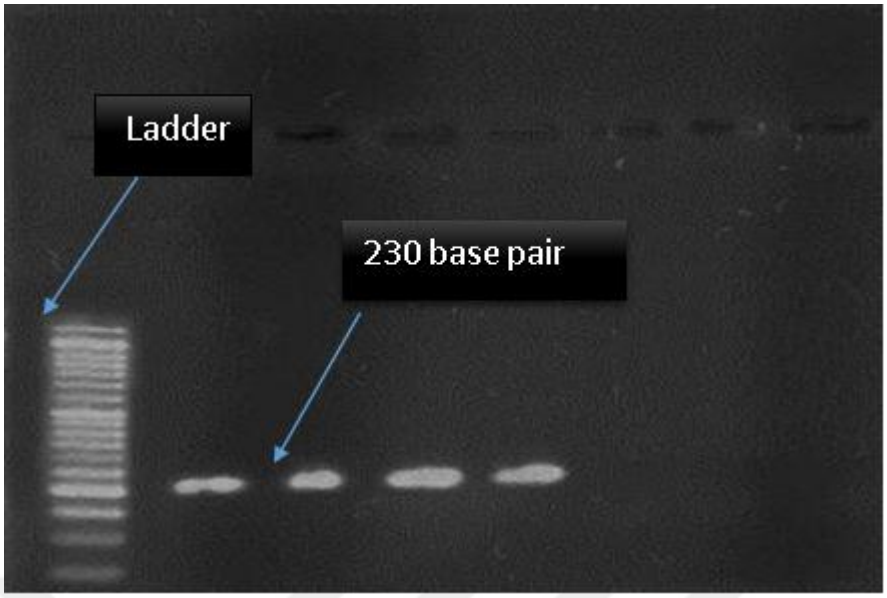

Figure 4.10 PCR product using abm master mix on 1.5 % agarose, 100 V, and for 60 minute (5 µL of DNA loaded in each well) ladder :50

4.7.2 The genotype distribution and the allele frequency for ABCG5 gene.

The ABCG5 gene was discovered on 2p21, the short (p) arm of chromosome 2 at location 21, within the human genes cluster. The ABCG5 gene, which has 15 exons, has a number of variants, some of which occur in the coding areas. Other mutations in the ABCG5 gene's noncoding regions have also been discovered (Pan *et al.* 2015).A substitution of guanine (G) by cytosine (C) leads in a polymorphism in the rs6720173 of the ABCG5 gene (C). This polymorphism was considered particularly important because it is located in exon 8, which is functionally distinct from other known exons. The Q604E polymorphism may affect ABCG5 gene translation by causing glutamine to be replaced by glutamic acid (Gln604Glu), thereby affecting final protein activity.The polymorphic allele that displays the restriction site of the BpuEI enzyme is referred to as Q allele, (Ting *et al.* 2016) which represents the most common allele, while the allele without the restriction site is referred to as E allele, which represents the minor allele, with a significant difference between the two allele ($p = 0.009$) and OR was 2.703 with CI (1.357-5.481) in patient compared to control group, as shown in Table 4.3, where the individual who carries E

Table 4.3 The allele frequency of ABCG5 (Q604E) gene polymorphism among study groups

-	CONTROL GROUP		PATIENT GROUP		OR (95% CI)		P-VALUE
Allele	Count	Proportion	Count	Proportion	-		
C	14	0.14	18	0.27	2.164(1.186-3.811)	2.703(1357-5.481)	0.009
G	86	0.86	82	0.73	0.838(0.717-0.966)		

OR: odds ratio, CI: confidence interval.

Homozygotes for the absence of the site are represented by the EE genotype, heterozygotes for the presence and absence of the site are represented by the QE genotype, and homozygotes for the showing of the site are represented by the QQ genotype.

As shown in Table 4.4, the QQ genotype was found in 25 (48 percent) of D.M patients, and this was similar to the frequency of patients who carried the QE genotype, which was also 23 (48 percent), while 2 (4 percent) of patients carried the EE genotype, which had the lowest frequency among DM subjects.

Within the healthy control group, the QQ genotype was found in 36 (74%) of healthy individuals, indicating a higher frequency among the control group, while the QE genotype was found in 14 (26%) of healthy individuals, indicating an intermediate frequency within the same group, and no individual had homologous genotype (EE) of rare allele (Tankova *et al*. 2001).

When compared to the healthy person group, patients have a greater frequency of the EE genotype, while controls have a lower frequency of the QQ genotype. Hardy-Weinberg proportions anticipated that the ABCG5 genotype distributions would be similar. A 2 percent agarose gel was used to separate the amplified PCR product, which

has a size of 240 base pairs. The electrophoresis cutting with BpuEI is shown in Figure 4.11.

Table 4.4 The genotype distribution of ABCG5 (Q604E) gene polymorphism among study groups

GROUP	GENOTYPE			TOTAL
	QQ (n%)	QE (n%)	EE (n%)	
Control	36 (74%)	14 (26%)	0 (0%)	50
Patient	25 (48%)	23 (48%)	2 (4%)	50
Total	61	37	2	100

If the restriction site of the enzyme is present, the BpuEI originate fragment of 1 base pair and 90 base pair after digestion

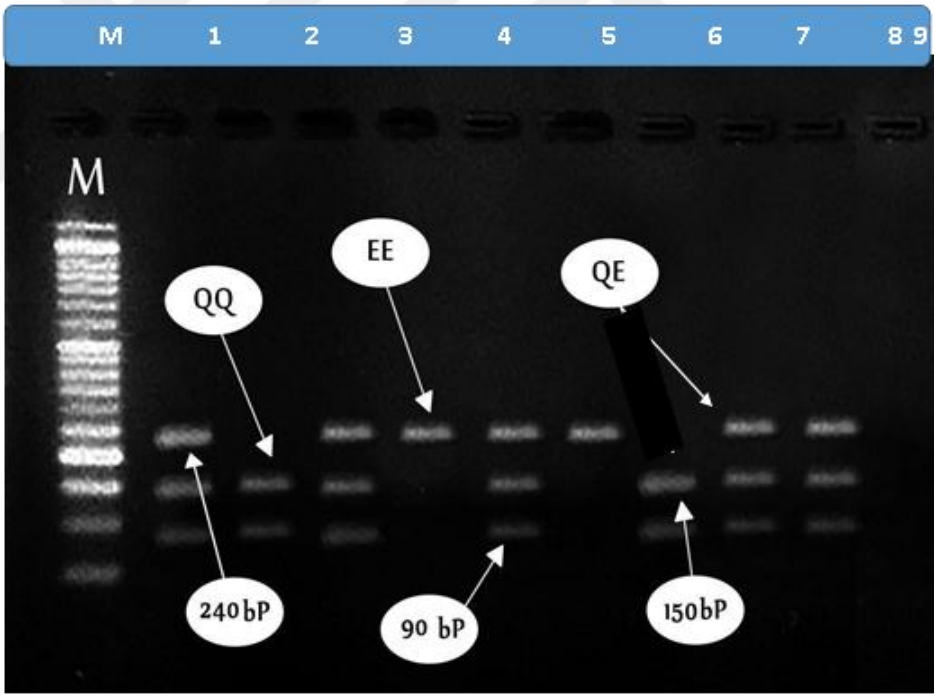

Figure 4.11 Agarose gel electrophoresis (2%) of the PCR product digested by the BpuEI endonuclease restriction enzyme BpuEI at 90 V/h, ladder: 50

Lane 4, 6: EE genotype homozygotes for absence of the site, 240 bp for patient individual.

Lane 1, 3, 4, 8, 9: QE genotype heterozygotes for the present and absence of the site, 240 bp, 150 bp and 90 bp for patient individual.

Lane 2, 7: QQ genotype homozygotes for the presence of the site, 240 bp for patient individual.

4.7.3 The determination association of ABCG5 genotypes with DM.

When compared to QQ genotype, the study found a significant link between QE genotype and DM people, with an odds ratio of 2.736, a p-value of 0.014, and a confidence range of 1.319-6.747 (Table 4.5). The odds ratio is the ratio of the number of times a specific event can occur to the number of times it cannot occur, and it is used to quantify the relationship between exposure (DM patients) and outcome (ABCG5 gene variant) (Ozougwu *et al.* 2013). This means that DM individuals have a two-to-one chance of having the QE genotype compared to non-DM individuals. When compared to QQ genotype, the study finds a significant connection between both EE and QE genotypes with DM patients (odd ratio 3.073, P-value= 0.008, confidence CI 1.330-7.249) (Vadhvaniya 2018.). This also means that patients are three times more likely than healthy people individuals to have both EE and QE genotypes. Where When DM patients were compared to controls, the minor alleles of Q604E polymorphisms were overexpressed. In the current study, the DM group had a 4% frequency of the EE genotype in the Q604E polymorphism, compared to controls group, which had 0% frequency of the same genotype. When compared to QE genotype, the association between QE, EE genotype with diabetes mellitus disease is not statistically significant (odd ratio=1.183, p-value = 0.869, confidence range 0.430-2.686) (Wu *et al.* 2014).

Table 4.5 Distribution of ABCG5 genotypes between patient and control group associated with carriage of Q604E polymorphism for statistical significance

ABCG5 GENOTYPES	ODDS RATIO	95%	CI
QE vs QQ	2.736	1.319 – 6.747	0.014*
EE and QE vs QQ	3.073	1.330 – 7.249	0.008**
QE and EE vs QE	1.183	0.430 – 2.686	0.869

*P < 0.05, CI = confidence interval.

Other studies have been referred to the significant association between E allele and gallstone disease, consequently Q604E considered as a major risk factor for incidence of DM diseases. levels than heterozygous QE genotype and the most frequent QQ genotype, which is associated with healthy individuals, and, although was non statistically significant.

When compared to the QQ genotype, the heterozygous QE genotype has higher levels of triglycerides, cholesterol, and LDLc but lower levels of HDL. However, also non statistically association was observed.

4.7.4 The genotype distribution and the allele frequency for ABCG8 gene.

These two genes, which are found on opposite strands, are separated by just 374 base pairs. It is hypothesized that these genes share a bidirectional promoter and regulatory elements because of their close proximity and opposing orientation (WHO 2016).

There are numerous variations in the ABCG8 gene, which includes 13 exons. To far, 42 variations have been discovered in ABCG8 genes. The most common types of variations are nonsense and missense, whereas splicing site variants are uncommon. The ABCG8 missense polymorphism (p.D19H, rs11887534) is connected to a gain-of-function that causes cholesterol-saturated lipoprotein hypersecretion. The D19H polymorphism in the ABCG8 gene is thought to raise the expression of ABCG8 or improve its activity, resulting in more effective cholesterol transfer into bile and cholesterol buildup, which is a major stage in the establishment of DM. as well as a considerable change in the protein's structure and function

The polymorphic allele that display the restriction site of the BCIVI enzyme referred to as H allele which represent minor allele, while the allele without the site of restriction referred to as D allele which represent in turn represented most frequent allele. PCR

product is performed for 100 sample, of which only 10 result was found, even after action of optimization. This result may be return to presence of variation in site of binding of primer from the population of paper that taken from it primer

The digestion for obtained pcr products showed found DD genotype which represent absence of restriction site, and DH genotype that indicate presence and absence of site, while HH genotype was represented with action of restriction enzyme showed in Figure 4.12

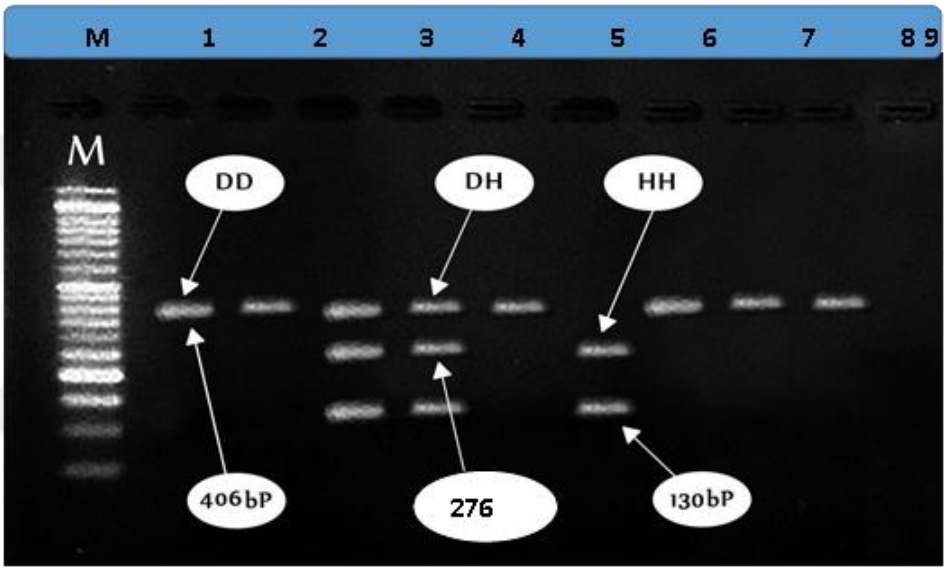

Figure 4.12 Restriction enzyme

Lane 1, 2,5,7,8,9: DD genotype homozygotes for absence of the site, 406 bp for patient individual.

Lane 3,4: DH genotype heterozygotes for the present and absence of the site, 406 bp, 276 bp and 130 bp for patient individual.

Lane 6: HH genotype homozygotes for the presence of the site, 276 bp and 130 bp for patient individual.

5 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

According to our results, the following can be concluded out of this study:

1. Carriers of ABCG5 604E polymorphism may have a two and half times risk for development of DM independent of age and body mass index.
2. The research shows that the EE and QE genotypes contributed to the disease's increased predisposition by acting as a transporter modulator as a consequence of the ABCG5 rs6720173 polymorphism, but the QQ genotype may protect against DM.
3. There were significantly reduced levels of ABCG5 and ABCG8 in the patients compared to the control group, suggesting that detecting these molecules might aid in estimating the risk of diabetes mellitus. In the QE genotype, there was a substantial reduction in the association between levels of ABCG5 proteins and the polymorphism of the ABCG5 gene.

This research also found no link between lipid profile and diabetes mellitus.

5.2 Recommendation

1. Further studied that evaluate effect of bothABCG5/ABCG8 genes on the opposite relation between DM disease and cardiovascular disease.
2. Study role of ABCG5/8 genes on Recurrence of DM after cholecystectomy.
3. don’t use primer of ABCG8 for Iraqi population, and perform sequencing to find out SNP variation.

REFERENCES

American Diabetes Association 2014a. Diagnosis and classification of diabetes mellitus. Diabetes care., 37: S81-S90.

American Diabetes Associationb. 2014. Standards of medical care in diabetes--2014. Diabetes care., 37: S14-S80.

Basher, A. S. 2017. design fuzzy control system for blood glucose level for type-1 diabetes melitus patients using GA a simulation study., 2017.

Benjamin, E. J., Blaha, M. J., Chiuve, S. E., Cushman, M., Das, S. R., Deo, R. and Muntner, P. 2017. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. Circulation., 135: e146-e603.

Boutayeb, A., Boutayeb, S. and Boutayeb, W. 2013. Multi-morbidity of non communicable diseases and equity in WHO Eastern Mediterranean countries. International journal for equity in health., 12: 1-13.

Buckley, B. S., Harreiter, J., Damm, P., Corcoy, R., Chico, A., Simmons, D. and DALI Core Investigator Group. 2012. Gestational diabetes mellitus in Europe: prevalence, current screening practice and barriers to screening. A review. Diabetic medicine., 29: 844-854.

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R. and Mirza, W. 2017. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Frontiers in endocrinology., 8: 6.

Dean, M., Hamon, Y. and Chimini, G. 2001. The human ATP-binding cassette (ABC) transporter superfamily. Journal of lipid research., 42: 1007-1017.

Dhatariya, K. K. and Umpierrez, G. E. 2017. Guidelines for management of diabetic ketoacidosis: time to revise?. The Lancet Diabetes and Endocrinology., 5: 321-323.

Díaz, A., López-Grueso, R., Gambini, J., Monleón, D., Mas-Bargues, C., Abdelaziz, K. M. and Borrás, C. 2019. Sex differences in age-associated type 2 diabetes in rats—Role of estrogens and oxidative stress. Oxidative Medicine and Cellular Longevity., 2019: ISO 690.

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 2004. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Spectrum., 17: 51-59.

Gomes, M. F., Amorim, J. B., Giannasi, L. C. and Salgado, M. A. C. 2018. Biomaterials for tissue engineering applications in diabetes mellitus. Biomaterials in Regenerative Medicine., 409.

Hadjadj, S., Cariou, B., Fumeron, F., Gand, E., Charpentier, G., Roussel, R. and Marre, M. 2016. Death, end-stage renal disease and renal function decline in patients with diabetic nephropathy in French cohorts of type 1 and type 2 diabetes. Diabetologia., 59: 208-216.

Hassan, M. 2018. Measurement of Prolactin Level among Type2 Diabetic Patients, Aldaraga Health Center, Wad Medani, Gezira State, Sudan 2017 Doctoral dissertation, University of Gezira., 2018.

Hayat, K., Gondal, M. A., Khaled, M. M., Ahmed, S. and Shemsi, A. M. 2011. Nano ZnO synthesis by modified sol gel method and its application in heterogeneous photocatalytic removal of phenol from water. Applied Catalysis A: General., 393: 122-129.

Hazard, S. E. and Patel, S. B. 2007. Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. Pflügers Archiv-European Journal of Physiology., 453: 745-752.

Joseph, M., Shyamasunder, A. H., Gupta, R. D., Anand, V. and Thomas, N. 2016. Demographic details, clinical features and nutritional characteristics of young adults with Type 1 diabetes mellitus-A South Indian tertiary center experience. Indian journal of endocrinology and metabolism., 20: 799.

Karalliedde, J. and Gnudi, L. 2016. Diabetes mellitus, a complex and heterogeneous disease and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrology dialysis transplantation., 31: 206-213.

Khunweeraphong, N., Mitchell- White, J., Szöllősi, D., Hussein, T., Kuchler, K., Kerr, I. D. and Lee, J. Y. 2020. Picky ABCG5/G8 and promiscuous ABCG2- a tale of fatty diets and drug toxicity. Febs Letters., 594: 4035-4058.

Kusuhara, H. and Sugiyama, Y. 2007. ATP-binding cassette, subfamily G (ABCG family). Pflügers Archiv-European Journal of Physiology., 453: 735-744.

Latif, S., Khan, M. Y., Qayyum, A., Qadir, J., Usman, M., Ali, S. M. and Imran, M. A. 2018. Mobile technologies for managing non-communicable diseases in developing countries. In Mobile applications and solutions for social inclusion (pp. 261-287).

Masharani, U. and German, M. S. 2011. Pancreatic hormones and diabetes mellitus. Greenspan's basic and clinical endocrinology., 8.

Mishra, S., Verhalen, B., Stein, R. A., Wen, P. C., Tajkhorshid, E. and Mchaourab, H. S. 2014. Conformational dynamics of the nucleotide binding domains and the power stroke of a heterodimeric ABC transporter. Elife., 3: e02740.

Mohan, V. and Anbalagan, V.P., 2013. Expanding role of the Madras diabetes research foundation-Indian diabetes risk score in clinical practice. Indian Journal of Endocrinology and Metabolism., 17: 31.

Ozougwu, J. C., Obimba, K. C., Belonwu, C. D. and Unakalamba, C. B. 2013. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol., 4: 46-57.

Pan, A., Wang, Y., Talaei, M., Hu, F. B. and Wu, T. 2015. Relation of active, passive, and quitting smoking with incident type 2 diabetes: a systematic review and meta-analysis. The lancet Diabetes and endocrinology., 3: 958-967.

Pham, N. M. and Eggleston, K. 2015. Diabetes prevalence and risk factors among Vietnamese adults: findings from community-based screening programs. Diabetes Care., 38: e77-e78.

Riddy, D. M., Delerive, P., Summers, R. J., Sexton, P. M. and Langmead, C. J. 2018. G protein–coupled receptors targeting insulin resistance, obesity, and type 2 diabetes mellitus. Pharmacological reviews., 70: 39-67.

Schwartz, S. S., Epstein, S., Corkey, B. E., Grant, S. F., Gavin III, J. R. and Aguilar, R. B. 2016. The time is right for a new classification system for diabetes: rationale and implications of the β-cell–centric classification schema. Diabetes care., 39: 179-186.

Selby, N. M. and Taal, M. W. 2020. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. Diabetes, Obesity and Metabolism., 22: 3-15.

Štefková, J., Poledne, R. and Hubáček, J. A. 2004. ATP-binding cassette (ABC) transporters in human metabolism and diseases. Physiol Res., 53: 235-243.

Suk, W. A., Ahanchian, H., Asante, K. A., Carpenter, D. O., Diaz-Barriga, F., Ha, E. H. and Landrigan, P. J. 2016. Environmental pollution: an under-recognized threat to children's health, especially in low-and middle-income countries. Environmental health perspectives., 124: A41-A45.

Tankova, T., Dakovska, L., Kirilov, G. and Koev, D. 2001. Insulin secretion and anti-GAD65 antibodies in subjects with impaired glucose tolerance. Experimental and clinical endocrinology and diabetes., 109: 355-360. ISO 690

Ting, D. S. W., Cheung, G. C. M. and Wong, T. Y. 2016. Diabetic retinopathy: global prevalence, major risk factors, screening practices and public health challenges: a review. Clinical and experimental ophthalmology., 44: 260-277.

Vadhvaniya, S. S. 2018. Microvascular Complications in Patients of Diabetes Mellitus. Doctoral dissertation, Sumandeep Vidyapeeth., 2018.

Vasiliou, V., Vasiliou, K. and Nebert, D. W. 2009. Human ATP-binding cassette (ABC) transporter family. Human genomics., 3: 1-10.

Wilson, C. A., Newham, J., Rankin, J., Ismail, K., Simonoff, E., Reynolds, R. M. and Howard, L. M. 2020. Is there an increased risk of perinatal mental disorder in women with gestational diabetes? A systematic review and meta- analysis. Diabetic Medicine., 37: 602-622.

World Health Organization and World Health Statistics 2016. monitoring health for the SDGs sustainable development goals. World Health Organization.

Wu, Y., Ding, Y., Tanaka, Y. and Zhang, W. 2014. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. International journal of medical sciences., 11: 1185.

Yu, X. H., Qian, K., Jiang, N., Zheng, X. L., Cayabyab, F. S. and Tang, C. K. 2014. ABCG5/ABCG8 in cholesterol excretion and atherosclerosis. Clinica Chimica Acta., 428: 82-88.

Zein, A. A., Kaur, R., Hussein, T. O., Graf, G. A. and Lee, J. Y. 2019. ABCG5/G8: a structural view to pathophysiology of the hepatobiliary cholesterol secretion. Biochemical society transactions., 47: 1259-1268.

Zhang, D. W., Graf, G. A., Gerard, R. D., Cohen, J. C. and Hobbs, H. H. 2006. Functional asymmetry of nucleotide-binding domains in ABCG5 and ABCG8. Journal of Biological Chemistry, 281 (7), 4507-4516.

Zhang, H., Huang, C. S., Yu, X., Lee, J., Vaish, A., Chen, Q. and Min, X. 2021. Cryo-EM structure of ABCG5/G8 in complex with modulating antibodies. Communications biology, 4 (1), 1-9. ISO 690

CURRICULUM VITAE

Personal Information

Name and Surname : Ammar Ali Hussein HWESHIM

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Chemistry	2020-2022
Undergraduate	University of Anbar College of Science Department of Chemistry	2005-2009