Republic of Iraq

Ministry of Higher Education and

Scientific Research

University of Kerbala

College of Pharmacy

Influence of Genetic Polymorphism of Organic Cation Transporter-1 on Therapeutic Response of Metformin in Type 2 Diabetes Mellitus Patients of Kerbala Province

A Thesis

***Submitted to the Council of College of Pharmacy/* University of Kerbala**
as Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology and Toxicology

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بسم الله الرحمن الرحيم

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Dedication

To my beloved family, Who have been my source of inspiration and gave me unconditional love, strength, and support every time.

To my sisters, relatives, friends, and colleagues who shared their advice and encouragement with me to go on.

To everyone who believes me and stood by my side by their support, and prayer to complete this thesis.

Halah

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Acknowledgements

First of all, I render my thankfulness to ALLAH who gave me a health, strength and ability to finish this study.

I would like to thank my first research supervisor ***prof. Dr. Ban Hoshi Khalaf***, for her continuous support, vast knowledge and patience.

I would like to thank my second supervisor ***Asst. prof. Dr. Hassan Mahmood Mousa Abo Almaali*** for his support, understanding, and his profound knowledge.

I would like to express my special thanks and gratitude to doctor ***Jabbar Fadeel Mahdi*** for his help and support to complete this thesis. And I am deeply grateful to ***Ass. Prof. Dr. Mazin Hamid Ouda AL-Rubaiee and Dr. Suzanne Jubair Abbas*****,** for their support during my laboratory work.

My thanks to **Professor Ahmed Salih Sahib**, the Dean of College of Pharmacy-University of Kerbela for his help. Also, I would like to express my deepest thanks to ***Assist. Prof. Amal Umran Mosa,*** the Head of Department of Pharmacology and Toxicology at University of Kerbala for her help.

I wish to express my thanks to all patients participating in this study and to all others who helped me in any way to complete this thesis.

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List of Abbreviations	
ADA	American Diabetes Association
AMPK	Adenosine 5-Monophosphate activated Protein kinase
ACC1	Acetyl-CoA carboxylase 1
AKT	AK strain Transforming
4-AAP	4-Aminoantipyrin
BMI	Body Mass Index
CHH	Congenital Hyperinsulinaemic Hypoglycemia
CVD	Cardio Vascular Disease
COX-I	Mitochondrial Complex I
CE	Cholesterol Esterase Enzyme
CHOD	Cholesterol Oxidase Enzyme
CI	Confidence interval
CRP	C- Reactive Protein
DM	Diabetes mellitus
DKD	Diabetic kidney Disease
DN	Diabetic nephropathy
DPP-4	Dipeptidyl peptidase-4
ER	Endoplasmic Reticulum
ELISA	Enzyme-Linked Immunosorbent Assay
Eag	Estimated Average Glucose
FPG	Fasting plasma glucose
FFA	Free Fatty Acids
FSG	Fasting Serum Glucose
FSI	Fasting Serum Insulin
GDM	Gestational diabetes mellitus
Glut4	Glucose transporter 4
GWAS	Genome-Wide Association Studies
GIP	Glucose-dependent Insulinotropic Polypeptide
GLP-1	Glucagon-like peptide 1 receptor agonists
GPD2	Glycerol 3-Phosphate Dehydrogenase
GPAT	Glycerol Phosphate Acyltransferase
HbA1c	Glycated haemoglobin
HGP	Hepatic Glucose Production
HMGR	β-hydroxy β-methylglutaryl-CoA reductase
HOMA-IR	Homeostasis model assessment-Insulin Resistance

HOMA-B	Homeostasis Model Assessment-Beta cells function
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
IR	Insulin Resistance
IDF	International Diabetes Federation
IRSs	Insulin Receptor Substrates
INSR	Insulin Receptors Tyrosine Kinase System
IHL	Intrahepatic lipid
IPL	Intrapancreatic lipid
IL-6	Interleukin 6
LKB1	Liver kinase B1
LDL	Low Density Lipoprotein
JNK	c-Jun N- terminal Kinase
MODY	Maturity-Onset Young Diabetes
MATE1	Multidrug And Toxin Extrusion 1
mTORC1	Mitochondrial complex 1
NDM	Neonatal Diabetes Mellitus
NEFAs	Non-Esterified Fatty Acids
NF-B	Nuclear Factor enhancer of activated B cells
NS	Non-Significant
OGTT	Oral Glucose Tolerance Test
OCT	Organic Cation Transporter
OR	Odd Ratio
PI3K	Phosphoinositide 3-kinase
PKB	Protein Kinase B
PKCβ	β isoform of protein kinase C
PMAT	Plasma Monoamine Transporter
POD	Peroxidase Enzyme
PCR	Polymerase Chain Reaction
R	Correlation coefficient
ROS	Reactive Oxygen Species
RPM	Round Per Minute
S	Significant
Syk	Spleen Tyrosine Kinase
SUR1	Sulfonylurea Receptor 1
SGLT2	Sodium dependent glucose co-transporter 2
SLC22	Solute Carrier 22
SNP	Single Nucleotide Polymorphisms
SD	Standard Deviation

T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TSC2	Tuberous Sclerosis Complex2
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
TTAB	Tetradecyl Trimethyl Ammonium Bromide
TC	Total Cholesterol
TG	Triglyceride
TNF	Tumor Necrotic Factor
UV	Ultra Violet
VAT	Vascular Adipose Tissue
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization
WAT	White Adipocyte Tissue

Abstract

Background

Diabetes is one of the most serious health problems in the world and the most common metabolic disease in humans, characterized by abnormal glucose and fat metabolism. The pathological abnormalities in type 2 diabetes mellitus (T2DM) are impaired insulin secretion from pancreatic beta-cell and/or impaired insulin action due to insulin resistance. The environmental and genetic factors consider important risk factors for T2DM. Multiple genes that contribute to T2DM susceptibility and in heterogeneity of drug responses which have been successfully identified by genome-wide association studies (GWAS).

Different responses to pharmacological treatment may be explained by the presence of a genetic mutation, These genetic variations are associated with either pharmacological targets or drug metabolism effects. Metformin is the first-line antidiabetic therapy for the treatment of T2DM over the past few decades, it acts primarily on tissues that targeted by insulin, such as the liver, muscle, and adipose tissues, by suppression of hepatic glucose production, increasing the glucose uptake by the skeletal muscles. Also decreasing the absorption of glucose in the intestinal mucosa, and reducing appetite.

The organic cation transporter (OCT1) is found mainly in human hepatocytes and it is responsible for the uptake of many endogenous and exogenous substances including metformin. Because the uptake of metformin by hepatocytes is necessary to elucidate its pharmacological

actions, This makes the OCT 1 very important for the therapeutic action of metformin

Variations in the SLC22A1 gene may lead to changes in the function of the OCT1 protein, resulting in a variety of metformin concentrations, these changes in pharmacokinetics and pharmacodynamics profile of metformin leading to change in the therapeutic response to it.

Aims:

To investigate the presence and distribution of Met420 deletion rs72552763 genetic polymorphism of OCT1 and its association with the pathogenesis of T2DM in diabetic male patients. And to investigate the effect of genetic polymorphism of OCT1 on therapeutic response to metformin male patients with T2DM in Kerbala .

Subjects and Methods:

In this cross-sectional study: a total of 150 healthy men and 200 male patients with T2DM treated with metformin (500 mg / tid) between the ages of 30 and 50 years were enrolled, all patients had already been diagnosed as diabetics with type 2 diabetes. Biochemical tests and genetic studies were performed for all patients and healthy control participants to determine the presence of OCT1 SNP and to analyze its effect on the therapeutic response of metformin.

Results:

The results demonstrated that the alleles frequencies of 420del OCT1 were similar in patients with T2DM and control group, concerning metformin action, patients with reference allele (wild type) and heterozygous allele of 420del OCT1 showed statistically different degrees of significant metabolic response to metformin, whereas the patients with mutant allele showed a less or statistically non-significant response.

Conclusion:

Genetic variation in OCT1 was associated with heterogeneity in response to metformin in male patients with T2DM in Kerbala, but it was not correlating with the incidence of type2 DM.

Chapter one

Introduction

1. Introduction

1.1 Diabetes Mellitus

Diabetes mellitus (DM) is a chronic condition that is widespread and poses a significant threat to human health. It is one of the leading causes of mortality worldwide and is a major cause of blindness, stroke, kidney failure, heart attacks, and lower-limb amputation.(1)

Its lifestyle disorder affects 8.3% of the world's adult population and rises at an alarming rate. The characteristic feature of diabetes is that the blood glucose is higher than the normal level which is caused by a defect in insulin secretion or impaired its action, or both. Diabetes can lead to chronic damage and dysfunction of various tissues, especially the eyes, kidneys, heart, blood vessels, and nerves.(2)

Diabetes mellitus may present with characteristic symptoms such as polyuria, thirst, weight loss, and blurred vision, It is associated with decreased life expectancy, major morbidity due to specific microvascular complications, increased risk of macrovascular complications (ischemic heart disease, stroke, and peripheral vascular disease) and decreased quality of life.(3)

Diabetes is a globally distributed disease that affects people of all ages, with prevalence rates that vary widely in different populations and within the same population. Epidemiological studies have shown that an increased frequency of life-style changes, urbanization, dietary changes, stress, and obesity are putative factors for glucose intolerance and diabetes mellitus.(4)

1.2 Types of Diabetes Mellitus

Four categories are generally included in Diabetes mellitus according to American Diabetes Association (ADA) which proposed the following classification.[5]

1. Type 1 diabetes mellitus (T1DM): is an autoimmune disease resulting in polyuria, polydipsia, weight loss, and hyperglycemia with progressive destruction of β-cells. T1DM requires lifelong administration of insulin. [6] The causes of the autoimmune destructive process of β -cells in T1DM are not fully explained but genetic and environmental effects have been involved, T1DM occurs mostly in children and adolescents.[7]

2. Type 2 diabetes mellitus (T2DM): is a result of insulin resistance (IR) and β-cell dysfunction, each of which is linked to the onset of DM independently. Although these conditions may overlap in some individuals, many individuals display either IR or β-cell dysfunction predominantly.[8]

3. Gestational diabetes mellitus (GDM): defined as "carbohydrate intolerance of varying degrees of severity with onset or first recognition during pregnancy" [8] . Glucose intolerance is often diagnosed from the second trimester onwards, but can also be detected in the first trimester of pregnancy in a small proportion of women, which may be representative of pre-existing pre-pregnancy glucose intolerance. Insulin or metformin may be used to achieve euglycemia if lifestyle changes failed.[9]

4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes. Monogenic diabetes is a group of relatively rare disorders that are caused by a single or several variants of genes. It represents a form of early-onset and non-autoimmune diabetes.[10] unlike Type1 and Type2 DM

Monogenic diabetes is primarily caused by genetics rather than the combined effect of genetic susceptibility and environmental factors. It is estimated that there is a monogenic cause for about 1-5 % of diabetes. So far, more than, 20 genes have been linked to monogenic diabetes. there are several forms of monogenic diabetes caused by highly penetrating single gene defects; the most common are the three main types of maturity-onset young diabetes (MODY), neonatal diabetes mellitus (NDM), and congenital hyperinsulinaemic hypoglycemia,(CHH).[6,11]

1.3 Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) has been referred to for a long time as non-insulin-dependent diabetes, or adult-onset diabetes characterized by insulin resistance, which could progressively worsen to absolute resistance, but in the past decade, the reduced β-cell function has been recognized as a key problem in T2DM.[5,12]

Type 2 diabetes mellitus is the major type of diabetes around the world. It is caused by the ineffective use of insulin by the body (Insulin Resistanse) with a slowly progressive loss of pancreatic β-cells. Although type1and 2 of diabetes may have the same symptoms, in T2DM they are often less marked or absent. So, it may be a silent disease without manifestation for a long time, until complications occur. For a long time this type of diabetes was observed only in adults, but, based on recent World Health Organization (WHO) data, it is also increasingly manifesting in children.[13,14]

1.3.1 Epidemiology of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus is the most prevalent form and is responsible for 90% of the overall diabetes prevalence than the other three major types of diabetes.(15)

According to the International Diabetes Federation (IDF) in 2019, diabetes caused 4.2 million deaths, and 463 million adults aged between 20 and 79 years old were living with diabetes, a number that will likely rise to 700 million by 2045. The greatest number of people suffering from diabetes are aged between 40 and 59 years old.(16)

The number of people with type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries, this dramatic increase in prevalence in both rural and urban settings, and affecting both genders proportionally.(17)

Between 2010 and 2030, the number of adults with diabetes mellitus in developed countries is projected to increase by 20 %, Asia has emerged as the major area with a rapidly developing T2DM epidemic. The top two epicenters of the global T2DM epidemic are China and India. The T2DM epidemic in these countries characterized by onset at a lower body mass index (BMI) and younger age than in Western populations.(18)

Six countries in the Middle East (Kuwait, Lebanon, Qatar, Saudi Arabia, Bahrain, and the United Arab Emirates) are among the ten countries with the highest prevalence of diabetes. In contrast to developed countries where the majority of people with diabetes are over retirement age, nearly three-quarters (73.4%) of diabetics in Arab countries are under the age of 60

years which are thus in their most productive years, further increasing the burden of disability due to diabetes.[19,20]

The prevalence in Iraq ranges from 8.5% to 13.9%, around 1.4 million Iraqis have diabetes.[13,21]

1.3.2 Clinical Presentation of Type2 Diabetes Mellitus

Type 2 diabetes usually develops in middle age or later, often after the age of 40, and is linked to a positive family history. Symptoms appear more slowly than in Type1, and the diagnosis is frequently made when an asymptomatic person's plasma glucose level is found to be elevated during a routine laboratory examination.[4]

The early stages of this disease are usually asymptomatic, or the symptoms are so mild that they go unnoticed. As a result, it is possible that it will go undiagnosed for many years, making an accurate estimate of the number of people who are affected extremely difficult. When symptoms present which include frequent urination, excessive thirst, hunger, fatigue, blurred vision, slow-healing wounds, and tingling, pain, or numbness in the hands and feet.[5]

Other signs and symptoms of type 2 DM include unexplained weight gain, pain, Acanthosis , paresthesia in the extremities, or a yeast infection, cramping, unusual drowsiness, frequent skin infections, dry, itchy skin and slow healing sores. The disease can also be accompanied by hypertension, dyslipidemia, polyphagia, and arteriosclerosis.[4,15,17]

Because T2DM patients are frequently asymptomatic, and the disease can go undiagnosed for years. Some studies have shown that typical T2DM

patients have had diabetes for at least 4-7 years at the time of diagnosis. Among patients with T2DM, 25% are described to already have retinopathy, 9% neuropathy and 8% nephropathy at the time of diagnosis.[15]

1.3.3 Diagnosis of Type2 Diabetes Mellitus

A diagnosis of diabetes has important implications for people, not only for their health, but also because of the potential stigma that a diabetes diagnosis may affect their employment, health and life insurance , driving status, social opportunities , and carry other cultural, ethical and human rights consequences.[22]

According to the American Diabetes Association (ADA), the diagnosis criteria of T2DM are as follow:[13]

1. Fasting plasma glucose (FPG) ⩾ 126 mg/dL (6.99 mmol/L).
2. Random plasma glucose ⩾ 200 mg/dL (11.1 mmol/L).
3. Two-hour plasma glucose ⩾ 200 mg/dL (11.1 mmol/L) 75-g an oral glucose tolerance test (OGTT)
4. Glycated haemoglobin (HbA1c) ⩾ 6.5% (48 mmol/mol).

The fasting plasma glucose and the 75-g oral glucose tolerance test both require individuals to fast overnight and the oral glucose tolerance test is time-consuming, uncomfortable and costly.[23]

Glycated haemoglobin consider an important indicator for long-term glycaemic control with the ability to reflect the cumulative glycaemic history of the previous 2–3 months, once a glucose molecule binds to hemoglobin in the red blood cells, hemoglobin remain glycated for the rest of its life-span (120 days).[11,24] In the measurement of HbA1c fasting is not

required but changes in red blood cell production rate or circulating life span will affect HbA1c levels, false HbA1c occurs in hemolytic anemia, blood loss, splenomegaly, and end-stage renal disease.[25]

1.3.4 Pathophysiology of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus has complicated pathophysiology. The two main pathological defects in T2DM are impaired insulin secretion due to pancreatic beta-cell dysfunction and impaired insulin action due to insulin resistance, with abdominal obesity being a major risk factor for the latter. Hyperglycemia, insulin resistance, and hyperinsulinemia have all been linked to a higher risk of complications in diabetic patients.[17,26]

Insulin resistance (IR) is defined as a decreased physiological response of tissues to insulin action, especially at the level of muscles, liver, and adipose tissues, with compensatory hyperinsulinemia that initially keeps plasma glucose levels within normal ranges. It's a hallmark of T2DM and cardiovascular disease, and it's linked to plenty of metabolic syndrome abnormalities and plays an important pathophysiological role in the development of diabetes.[27]

Insulin resistance is established by genetic and environmental factors, whereas genetic mutations are causes of rare and severe insulin resistance, obesity can cause insulin resistance through a variety of mechanisms .[28]

Insulin resistance in skeletal muscle is a major pathogenic factor in T2DM, the principal function of insulin in the skeletal muscle is to promote cellular glucose uptake, a process that contained a cascade of events started with insulin binding to its cell surface receptor, followed by receptor

autophosphorylation and activation of receptor tyrosine kinases, resulting in tyrosine phosphorylation of insulin receptor substrates (IRSs) such as IRS1, IRS2, IRS3, and IRS4, binding of IRSs to the regulatory subunit of phosphoinositide 3-kinase (PI3K), results in activation of PI3K and Akt/protein kinase B (PKB), this complex activate and stimulates the translocation of insulin-mediated glucose transporter 4(Glut4) which is the major insulin-regulated glucose transporter from intracellular vesicles to the plasma membrane and transverse tubules.(29,30) Mutations that reduce the expression of insulin receptor or GLUT4, as well as any defect or mutations in any of the main phosphorylation sites of insulin receptors tyrosine kinase system (INSR) or key proteins of the downstream signaling pathway such as IRS-1, and IRS-2 or phosphoinositide 3-kinase (PI3K) this would reduce glucose intake and impair insulin action on the muscle resulting in a hyperglycaemic state.(16,31)

The liver perform a unique role in glucose metabolism and it is essential for systemic glucose homeostasis, the main processes in the liver that contribute to glucose homeostasis, include glycogen synthesis, glycolysis, glycogenolysis, and gluconeogenesis, all of which are regulated by pancreatic endocrine cell hormones, glucagon, and insulin. Under hyperglycemic and hyperinsulinemic conditions of insulin resistance in T2DM the arterial blood glucagon- insulin ratio dramatically changed and the net hepatic glucose balance changed also, so patients with T2DM show impaired liver glucose uptake and increased hepatic glucose production (HGP).(32,33)

In white adipocyte tissue (WAT) insulin control plasma levels of non-esterified fatty acids (NEFAs) for maintaining euglycemia; insulin

suppression of lipolysis is a key physiological function in WAT. Insulin suppresses plasma NEFA levels by rapidly inhibiting triglyceride lipolysis. Also, Insulin-stimulated glucose uptake in the adipocyte, through IRS1-PI3K-AKT axis.[34] Adipose IR refers to adipose tissue's impaired response to insulin stimulation (Adipose-IR). So adipose-IR can result in impaired lipolysis suppression, impaired glucose uptake, and increased free fatty acids (FFA) release into plasma.[16]

Despite the diseases' genetic roots, the prevalence of type 2 diabetes is rising at a faster rate than genetic variation, implying that environmental factors play a role in diabetes. Environmental factors such as diet, endocrine disruptors, and other environmental polluters, as well as gut microbiome composition, are all linked to type 2 diabetes. Obesity also plays a main role in insulin resistance and type 2 diabetes.[35] The increase in visceral adipose tissue leads to higher levels of FFA, which activates the β isoform of protein kinase C (PKCβ) and inhibit glucose transport-4 (GLUT-4) translocation to the cell membrane, and also disrupt insulin signal transmission by phosphorylating IRS-1 on serine/threonine residues rather than on tyrosine. all that leads to insulin resistance .[36]

In T2DM β -cell dysfunction may be caused by a complex network of interactions between the environment and various molecular pathways implicated in cell biology, in an excessive nutritional state similar to that found in obesity, hyperglycemia, and hyperlipidemia are often present, under these circumstances, β -cells are exposed to toxic pressures such as inflammation, inflammatory stress, endoplasmic reticulum(ER) stress, metabolic/oxidative stress, and amyloid stress, which leads to a loss of islet integrity.[16] The pancreatic lipid accumulation also had a role in developing

T2DM through a release of free fatty acid and lipid intermediates which interfering with cell signaling and causing beta-cell apoptosis by a process called lipotoxicity, the level of intrahepatic lipid(IHL), intrapancreatic lipid(IPL) and vascular adipose tissue (VAT) are inversely associated with beta-cell function and insulin secretion.[37]

In human obesity and type 2 diabetes, pancreatic islet cells show signs of inflammation, including immune cell infiltration and increased expression of cytokines (IL-19, IL-25, CSF1) and chemokines (CCL5, CCL7). All this may be responsible, at least in part, for β cell dysfunction, death, and insulin secretion dysfunction.[38,39] In addition, increased production of reactive oxygen species (ROS), driven by chronic hyperglycemia and hyperlipidemia, is thought to be a major cause of beta-cell dysfunction in diabetes. ROS damage the cellular machinery components in pancreatic cells such as DNA, proteins, and lipids, resulting in a wide range of harmful effects. Furthermore, compared to other tissues, pancreatic islets, particularly beta-cells, express fewer antioxidant enzymes, making them more vulnerable to the harmful effects of oxidative stress and reactive oxygen species. [40,41]

1.3.5 Risk Factors for Type2 Diabetes Mellitus

Many risk factors have the potential to increase the probability of a person developing T2DM.

Risk factors for T2DM are divided into 1- modifiable risk factors such as physical inactivity, overweight and obesity, abnormal cholesterol (lipid) levels and high blood pressure (hypertension) 2- non-modifiable risk

factors as socioeconomic demographic characteristics as well as genetics, age, mental health, and history of gestational diabetes.[42,43]

Physical inactivity is an important risk factor in the development of T2DM. Physical activity is associated with a significant decrease in the development of T2DM, due to an increase in insulin sensitivity. During physical activity contracting skeletal muscle increases glucose uptake into the cells; physical activity also increases blood flow in the muscle and promotes glucose transport into the muscle cells, also it has been reported to decrease intra-abdominal fat, a major risk factor for insulin resistance and T2DM development.[44]

Obesity one of the major contributors to T2DM pathogenesis leads to rapid fat cell expansion in both cell size and number with oxygen demand exceeding the supply. This low-oxygen state (hypoxia) causes inflammation of the tissue and prevents the normal response of fat cells (adipocytes) to insulin.[45,46] Many cellular and molecular evidence support obesity as a condition of sub-acute chronic inflammation, this inflammatory state is thought to reduce insulin responsiveness in insulin-sensitive tissues and increasing the risk of T2DM.[47]

Type 2 diabetes mellitus usually affects individuals over 40 years of age.[48] Aging leads to an increase in the prevalence of diabetes and carbohydrate intolerance in the elderly due to a decrease in the secretion of insulin in response to glucose load and increased resistance to insulin in peripheral tissues.[49]

Multiple genes that contribute to T2DM susceptibility have been successfully identified by genome-wide association studies (GWAS). [50] about 250 genetic variants have been identified contribute to the risk of T2DM.[11] The complexity for evaluating the contribution of genetic factors to T2DM arises from the impact of other risk-related family and behavioral factors, including obesity, decreased physical activity, and dietary habits. Although the overall risk of T2DM appears "more heritable" than type 1 diabetes.[51,52] Several studies show that if one of the family members has T2DM, the chances of developing this condition will increase .[42] For people whose one of parents are diagnosed with T2DM, the possibility of developing T2DM is 40 % and more than 60 % if they have two diabetic parents.[53]

Three major contributors lead to varying insulin sensitivity between men and women; estrogen activity, differences in the distribution of adipose tissue, and adipokine secretion.[54] The risk of developing T2DM and the prevalence of this condition are associated with lifestyle differences related to gender. Females with elevated body mass index (BMI) tend to be more at risk for T2DM than males with elevated BMI, and mothers are more likely than fathers to transmit T2DM to their children.[42] Insulin resistance and estrogen deficiency are two disorders that are related to each other based mainly on defective cellular glucose uptake and on an atherogenic serum lipid profile. .Moderate to severe estrogen deficiency was found to be a risk factor for insulin resistance in both premenopausal and postmenopausal women. Insulin resistance and hyperinsulinemia, on the other hand, cause a severe imbalance in sexual steroid synthesis, resulting in high androgen

levels and low estrogen levels, as well as interfering with estrogen effect even at receptor levels .[55]

Smokers are more likely than nonsmokers to have central fat accumulation, and smoking is known to cause insulin resistance and compensatory insulin secretion responses, which could explain why smokers have a higher risk of T2DM. Current smokers had a 45 % higher risk of T2DM than non-smokers, and there was a dose–response relationship between the number of cigarettes smoked and the risk of T2DM.[18]

Some medications like Glucocorticoids, some HIV medications, and atypical antipsychotics have all been linked to an increased risk of type2 diabetes.[56]

Economic stability and education are two of the social determinants of health that are significantly associated with diabetes status .[54] T2DM risks are increasing for people with lower education and economic status, including immigrants. T2DM prevalence, incidence, and mortality rates are much higher among migrants than among the native population in host countries.[57]

Omentin-1, an important adipokine, is secreted by visceral fat adipose tissue and acts in a variety of ways, including endocrine, paracrine, and autocrine. There are several hypotheses suggested that the alteration in omentin-1 secretion could affect glucose homeostasis, and thus contribute to the development of obesity and diabetes.[58,59] The AKT signaling pathway is activated by omentin, which increases insulin-stimulated glucose uptake in human adipocytes by increase insulin signal transduction.[60] In humans, there is an inverse relationship between omentin levels and IR. Also,

omentin has anti-inflammatory properties and could lower cytokine expression.[16]

Insulin resistance, inflammatory processes, endothelial dysfunction, and cardiovascular diseases are thought to be linked to abnormal omentin-1 secretion. Many studies found that the level of omentin -1 was reduced in patients with impaired glucose tolerance and T2DM, also fasting serum omentin-1 levels were found to be negatively related to insulin level and HOMA-IR in T2DM patients. [61]

1.3.6 Complications of Type2 Diabetes Mellitus

Diabetes complications are a leading cause of death, such complications lead to increased disability, lower quality of life, and increased use of healthcare services, putting more pressure on both individuals and healthcare systems. Thus, trying to maintain euglycemia is critical to avoiding these complications, which include severe coronary heart disease, kidney failure, foot ulcers, and blindness.[62,63]

Complications of diabetes can affect multiple organ systems, presenting in various ways for different people. Undiagnosed diabetes and poor glycemic control represent a significant health problem and the main leading causes of complications of T2DM.[63]

In people with T2DM, vascular complications of both the macrovascular (cardiovascular disease (CVD)) and microvascular (diabetic kidney disease (DKD), diabetic retinopathy, and neuropathy) systems are the leading cause of morbidity and mortality.[64]

Diabetes mellitus has a twofold to tenfold increased risk of a cardiovascular event independently on age, BMI, smoking status, or even chronic hypertension. [18] The metabolic syndrome, which includes hyperglycemia, insulin resistance, and dyslipidemia, as well as hypertension and central obesity, may play a role in the development of atherosclerosis which is a major cause of heart disease and stroke in T2DM patients.[65] Hyperglycemia in type2 DM is known to stimulate the spleen tyrosine kinase (Syk) system, which inhibits mitochondrial complex I (COX-I) activity and expression , this process inevitably leads to an increase in cellular and/or mitochondrial ROS production, ROS will induce atherosclerosis through activation of the c-Jun N- terminal Kinase (JNK) stress signaling pathway, endoplasmic reticulum (ER) stress as well as cardiomyocyte death and endothelial cell dysfunction via apoptosis. Another contributing factor that leads to endothelial cell dysfunction and development of atherosclerosis is glycation of all proteins, particularly collagen crosslinking and matrix proteins of the arterial wall, which is also caused by persistent hyperglycemia.[66,67]

Obesity and hyperlipidemia represent sub-acute chronic inflammation conditions associated with the activation of at least two major inflammatory pathways, stress-activated JNK, and the transcription factor nuclear factor enhancer of activated B cells (NF-B), with the involvement of activated monocytes and tissue macrophages amplifying the inflammatory state through the production of pro-inflammatory cytokines and promote the risk of atherosclerosis in type2 DM .[47,68]

Diabetic nephropathy (DN) is a major chronic microvascular complication of diabetes mellitus and a leading cause of kidney failure. Poor glycaemic control, hypertriglyceridemia, increased production of cytokines like interleukin 6 (IL-6) and tumor necrosis factor all represent the pathogenesis factors for inflammation in type2 DM. These factors are cytotoxic to glomerular epithelial cells, and protein permeability barrier of the glomerulus and lead to increase production of ROS, increase renal sodium reabsorption and decrease erythropoietin production, the final results of that is renal damage and changes in hemodynamic factors of renal cells. [44,69]

Diabetic nerve damage (neuropathy) is common, especially in the longer peripheral nerves that innervate the lower limbs. [64] Diabetic neuropathy is recognized by the presence of symptoms and/or signs of peripheral nerve dysfunction in patients with type2 DM. Sensory, focal/multifocal, and autonomic neuropathies are all possible manifestations of peripheral neuropathy in diabetes.[3]

Diabetic retinopathy is caused by the damage of small blood vessels (microangiopathy) and the growth of friable and poor-quality new blood vessels in the retina, and macular edema, which can result in severe vision loss or blindness in T2DM.[4]

Elevated oxidative stress caused by hyperglycemia can cause retinal basement thickening, which manifests as microangiopathy in diabetic retinopathy. Furthermore, because retinal tissue is high in lipids, it is especially vulnerable to oxidative stress. Increased vascular leakage and

permeability result from increased oxidative stress in the retina, resulting in diabetic macular edema.[67]

1.3.7 Treatment of Type 2 Diabetes Mellitus

The goals of the treatment of T2DM is to control blood sugar, enhance insulin sensitivity, improve insulin resistance, alleviating the symptoms, and reduce the risk of micro and macrovascular diabetic complications.[70]

There are two ways for the treatment of T2DM, 1-by using the non-pharmacological way like a healthy diet, exercise, and good lifestyle management, this way can help to a certain extent to improve insulin sensitivity. However with the development of the disease the patients will need 2- pharmacological medication like oral hypoglycemic agent (OHA) and maybe insulin injection to control blood sugar.[11]

1.3.7.1 Non-Pharmacological Treatment

Changing the lifestyle through targeted changes in behavior, including weight loss and physical activity, can reduce the glycemic index, blood pressure, and levels of lipids. Additional control measures are also required, such as proper control of blood pressure and lipid reduction, to reduce cardiovascular risk and other complications.[71]

Evidence from randomized controlled trials in Europe and the USA has shown that lifestyle modifications including healthy diet counseling and regular physical activity can reduce the risk of diabetes in high-risk individuals over a period of 3-5 years by 58%. The American Diabetes Association (ADA) recommends intensive behavioral lifestyle intervention programs for pre-diabetes including increase moderate-intensity physical

activity to at least 150 min/week to achieve and maintain a 7% loss of initial body weight.[57]

1.3.7.2 Pharmacological Treatment

There are numerous types of medications recommended for T2DM management, each type of medication works in a unique way to maintain the blood glucose levels in the normal target range as shown in table (1-1) [11]

Table (1-1) Summary of some antidiabetic drugs [72]

Antidiabetics	Mechanisms of action
α-glucosidase inhibitors (Acarbose, Miglitol)	Inhibit carbohydrates degradation in intestinal villi
Biguanides (Metformin)	Block liver gluconeogenesis, skeletal muscle uptake of glucose, ↓the absorption of glucose in the intestinal mucosa,↑ plasma levels of GLP-1
Dipeptidyl peptidase-4(DPP-4) inhibitors (Sitagliptin, Saxagliptin)	↑ incretin (glucose-dependent insulinotropic polypeptide GIP and GLP-1) concentrations, ↑ insulin secretion, ↑glucagon secretion
Exogenous insulin	Activates the insulin receptor,↓ hepatic glucose output

Glucagon-like peptide 1 receptor agonists (GLP-1) (Exenatide, Liraglutide)	Binds to GLP-1 receptor, causing: ↑ insulin secretion, delayed gastric emptying, and satiety
Meglitinides (Nateglinide , Repaglinide)	Binds to B-cell sulfonylurea receptor 1(SUR1)
Sulfonylureas (Glibenclamide, Glimepiride)	↑ insulin secretion by activating B- cell SUR 1
Sodium dependent glucose co-transporter 2 SGLT2 inhibitors Dapagliflozin, Canagliflozin	Limits renal glucose reabsorption
Thiazolidinediones (Pioglitazone, Rosiglitazone)	Activators of the peroxisome proliferator-activated receptor ϒ,↑ differentiation of pre-adipocytes,↑ insulin sensitivity in muscle, hepatic and adipose tissue,↑glucose uptake in peripheral tissues

1.4. Metformin

Based on the official guidelines of the ADA, metformin has been recommended as the first-line antidiabetic therapy for the treatment of T2DM over the past few decades and it has been widely used since the 1950s. Metformin, an insulin sensitizer, acts primarily on tissues that target insulin, such as the liver, muscle, and adipose tissues.(73,74)

Metformin is a synthetic biguanide belonging to a hydrophilic base existing at physiological pH in cationic form and with minimal passive membrane diffusion (70,75), In particular, metformin suppresses hepatic gluconeogenesis without increasing the burden of pancreatic β-cells to increase insulin secretion or promote weight gain induction by adipocyte differentiation. However, it remains unclear the exact molecular mechanisms of its glucose-lowering effects.(76)

The most significant adverse drug reactions of metformin are lactic acidosis, hypersensitivity reaction, vitamin B12 deficiency, altered taste, and gastrointestinal intolerance. 20–30% of people treated with metformin develop gastrointestinal side effects, and 5% are unable to tolerate metformin due to the severity of these side effects.(73) The gastrointestinal side effects of metformin include the following symptoms during metformin therapy: bloating, abdominal pain, nausea, diarrhea, vomiting, and anorexia, in the absence of any acute gastrointestinal disease. However, It has a number of advantages over other Type 2 diabetes treatments, including low risk of hypoglycaemia, weight neutrality, low cost and possible cardiovascular benefits .(75)

1.4.1 Metformin Pharmacokinetics

Metformin is orally administered, has low bioavailability (40%–60%). The absorption of metformin occurs in the upper small intestine, with insignificant absorption in the large intestine. The drug's plasma half-life is between 4 and 8 hours in people who don't have kidney disease. Metformin is distributed within 6 hours after absorption and not bound to plasma proteins, metformin is not metabolized and is excreted unchanged in the urine by passive glomerular filtration and active renal secretion.(77,78)

Metformin is an unusual hydrophilic drug that, under physiological conditions, mostly exists in a positively charged protonated form. Rapid and passive diffusion through cell membranes is unlikely due to these physicochemical properties. The transport of metformin involves an active uptake process through solute carrier organic cation transporters, which is one of the major transporters known to play an important role in the pharmacokinetics of metformin to date.(79,80)

After oral administration metformin is absorbed into the enterocytes via the apical membrane's plasma monoamine transporter (PMAT) and organic cation transporter 3 (OCT3), and it leaves the enterocytes via the basolateral membrane's OCT1. Then metformin is delivered directly to the liver via the portal vein. Metformin enters the liver via the OCT1/3 transporter on hepatocyte membranes, and it is excreted from hepatocytes via the multidrug and toxin extrusion 1 (MATE1) transporter. Then metformin is transport from the bloodstream into renal epithelial cells by OCT2 and excreted in urine by MATE1/2k.(81–83)

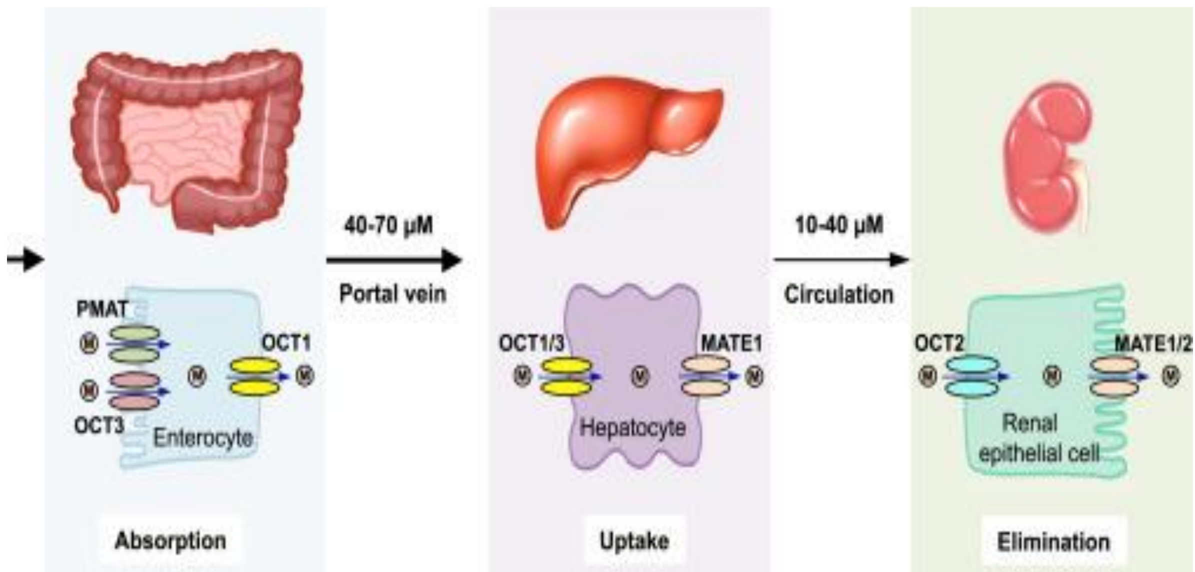


Figure (1-1): Main actions of metformin transporters.[82]

1.4.2 Metformin Mechanisms of Action

Due to its glucose-lowering effects, which are mediated through the suppression of hepatic glucose production, metformin is the most commonly prescribed medication for T2DM. Metformin also increases the glucose uptake by the skeletal muscles, decrease the absorption of glucose in the intestinal mucosa, and reduces appetite.[72,84]

Several mechanisms for the action of metformin on hepatic gluconeogenesis and the production of glucose have been identified in the past decade. An important breakthrough was that metformin could activate adenosine 5-monophosphate activated protein kinase (AMPK), a master regulator of various metabolic pathways by increasing its phosphorylation at Thr-172, by a mechanism known as AMPK- dependent mechanism.[76]

Activation (phosphorylation) of AMPK reduces glucagon-mediated glucose output by the liver and suppresses hepatic gluconeogenesis.[85] Also, metformin acts as a non-competitive inhibitor for mitochondrial glycerol 3-phosphate dehydrogenase (GPD2), this inhibition leads to impaired respiration, decreased cytoplasmic NAD+/NADH ratio, and undermined glucose production from both glycerol and lactate by a mechanism known as AMPK- independent mechanisms of metformin.[86]

Treatment with metformin leads to stimulate glucose transporter-4 (GLUT4) translocation in muscle and fat tissues after activation of the (AMPK) which activates insulin receptor(INSR) and insulin receptor substrate 1 (IRS1) leading to increase insulin sensitivity along with decreased liver gluconeogenesis.[73,87]

Metformin also affects the mTORC1 pathway, the master regulator of protein synthesis. Metformin inhibits the mTORC1 complex by activating the tuberous sclerosis complex 2(TSC2) the negative regulator of mTORC1 and phosphorylating raptor, a subunit that inhibits mTORC1activity.[86]

Metformin accumulates inside the mitochondria and inhibits complex I to prevent mitochondrial ATP production and thus raising the ADP:ATP and AMP:ATP ratios in the cytoplasm. AMPK is activated as a result of these changes.[88,89]

Moreover, the pharmacological reactivation of downregulated AMPK in fatty liver is adequate to normalize the lipid content of the liver. Mechanistically, AMPK activation reduces hepatic triglyceride content both by impairing lipid synthesis and by promoting fatty acid oxidation in a liver kinase B1 (LKB1) dependent way.[90] The reactivation of AMPK could

inhibit the conversion of acetyl-CoA to malonyl-CoA by phosphorylation of acetyl-CoA carboxylase 1 (ACC1) and ACC2, as a result, metformin decreased liver lipogenesis and hepatosteatosis.(76) AMPK also inhibits the synthesis of cholesterol and phospholipid/triacylglycerol, the former by phosphorylation and inactivation of β-hydroxy β-methylglutaryl-CoA reductase (HMGR) and the latter by suppression of glycerol phosphate acyltransferase (GPAT).(91)

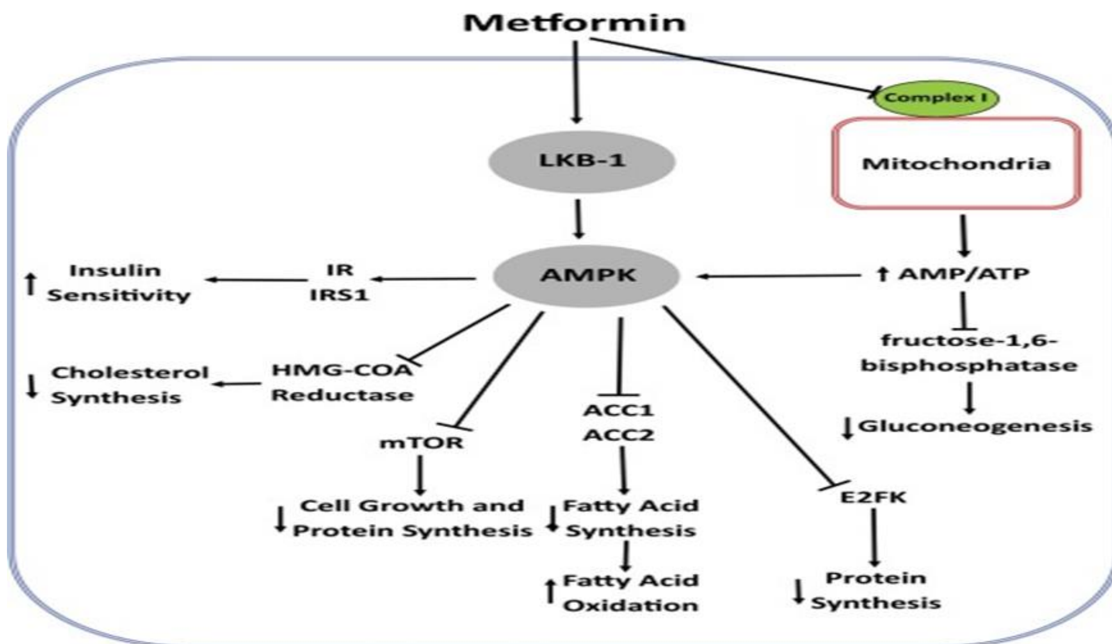


Figure (1-2): The pathways in which metformin affects the metabolism of cells.(92)

1.5 Organic Cation Transporters

The organic cation transporters (OCTs) are members of the solute carrier 22 (SLC22) family, which includes 65 SLC families (SLC1–65) with over 400 genes. SLCs regulate the transport of most of the molecules required for cell survival across biomembranes, and they have been linked to a hundred monogenic disorders.[93] The most important OCT1 (SLC22A1) and OCT2 (SLC22A2) are primarily expressed in hepatocytes and proximal tubular cells of the liver and kidney, respectively, whereas OCT3 (SLC22A3) is highly distributed in astrocytes, the blood-cerebrospinal fluid barrier in choroid plexus epithelial cells, as well as the placenta, bronchial, and intestinal epithelium.[94]

Polyspecific transporters OCTs recognize and transport a wide range of molecules with positively charged or zwitterions at physiological pH like organic amines choline, carnitine, neurotransmitters like dopamine and serotonin, microbiota products like trimethylamine (TMA), trimethylamine N-oxide (TMAO), and vitamin B1 thiamine. It also transports drugs like anticancer platinum derivatives, ifosfamide, antibiotics like gentamicin, cephaloridine, colistin, and antidiabetic drugs like metformin.[93]

Organic cationic transporter 1 is one of three polyspecific cationic transporters that mediate the uptake of many organic cation of endogenous and exogenous substances from the blood into epithelial cells.[95] It is encoded by the gene of the solute carrier family 22 members 1 (SLC22A1). SLC22A1 is a gene on chromosome 6 as 6q26 that is found in a cluster. It consist of 11 exons and 10 intron and spans about 37kb.[96,97]

The SLC22A transporter family members consist of 12 α-helical transmembrane domains with an extracellular N-glycosylated loop between domains 1 and 2. While the large intracellular loop with the designated phosphorylation sites is found between the 6th and 7th domain[98] as shown in (figure 1- 3)

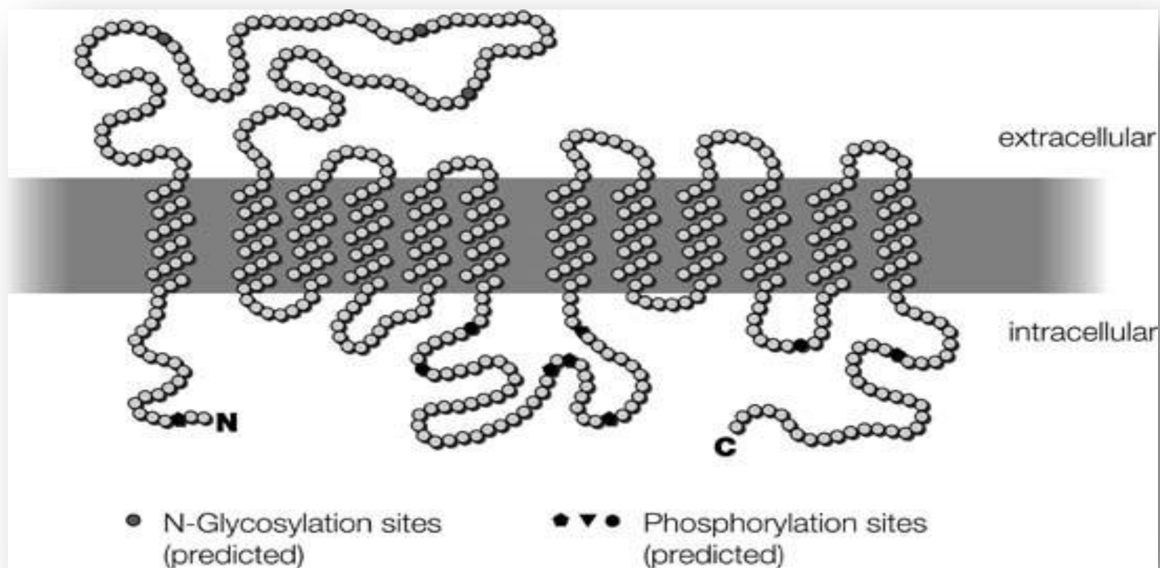


Figure (1-3): General topology of organic cationic transporter.[98]

Organic cation transporter 1 is a 553-amino-acid protein. OCT1 is primarily expressed in the liver, but it is also found in other tissues, implying that it serves as a housekeeping function in the body. OCT1 is expressed at the sinusoidal (basolateral) membrane of hepatocytes in the liver, where it mediates organic cation uptake. OCT1 is also found in the apical membrane of the trachea and bronchi, as well as in neurons, where it aids in the

maintenance of the electrochemical gradient in the blood-brain barrier, immune cells, and kidney.[98,99]

The pharmacokinetics and pharmacodynamics profile of metformin are mediated mainly by OCT1 and OCT2 because they are responsible for the intestinal absorption, hepatic uptake, and renal transport,[70,100,101] and because the uptake of metformin by hepatocytes is necessary to elucidate its pharmacological actions, this makes the OCT 1 very important for the therapeutic action of metformin.[102,103]

1.6 Effect of Organic Cation Transporter 1 Polymorphisms on the Therapeutic Response of Metformin

The SLC22A1 gene is highly polymorphic, and there are numerous single nucleotide polymorphisms (SNP) that have been reported to be associated with its activity. This may clarify some of the inter-individual variabilities in the efficacy of metformin.[98,104,105] Variations in the SLC22A1 gene have led to changes in the function of the OCT1 protein, resulting in a variety of metformin plasma concentrations and a reduction in metformin levels, leading to decreased in the therapeutic response to metformin.[106,107] Polymorphisms of OCT1 also are correlated with diseases like diabetes mellitus, diabetic nephropathy, primary biliary cirrhosis, and hypertension.[97]

The OCT1 polymorphisms may lead to a lower therapeutic impact of metformin, and besides that, it may have been associated with metformin's digestive side effects as OCT1 is expressed in enterocytes and

their genetic variations can affect metformin's intestinal uptake and cause gastrointestinal side effects.[53] The biological mechanism for gastrointestinal intolerance to metformin remains poorly understood, but the exposure to higher concentrations of metformin in the gastrointestinal tract as a result of reduced- function of OCT1 can partially explain the risk of metformin-induced gastrointestinal side effect.[108,109]

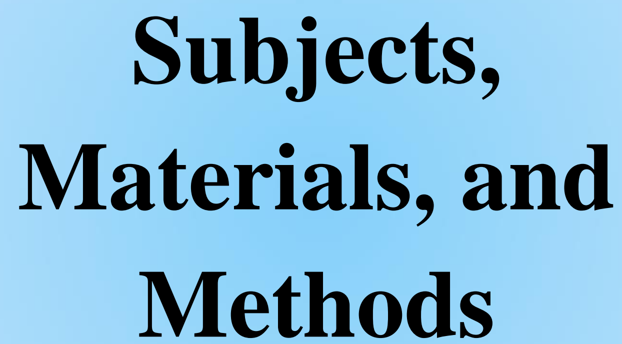
According to previous studies there are a total of 34 OCT1 polymorphisms were identified in 10 ethnic groups with highly differences in the frequencies of common alleles were founded among these groups ,each one of these polymorphisms have been associated with different deleterious effects on metformin response.[110,111]

One of most common variant of OCT1 gene is Met420 deletion rs72552763, which occurs in exon 7, it is a deletion of three bases 3bp (ATG) at codon 420. The deletion variant of Met420 is quite common and has a frequency in Caucasians of ~18.5 % and in African Americans of 5 %. This variant is associated with reducing hepatocyte metformin uptake by about (60%) with digestive side effects and metformin intolerance.[106,112]

1.7 Aims of Current Study

1. To investigate the presence and distribution of genetic polymorphism of organic cation transporter 1 (OCT1) and its association with the pathogenesis of type 2 diabetes mellitus in male patients in Kerbala province.
2. To investigate the effect of genetic polymorphism of organic cation transporter 1 on therapeutic response to metformin in male patients with type 2 diabetes mellitus in Kerbala province .

Chapter Two



Subjects, Materials, and Methods

2. Subject, Materials, and Methods

2.1 Subjects (Patients and Control)

A total of 200 male patients aged 30-50 years were enrolled in this study when they visit a private clinic to get medical treatment and advice about their cases. This study was performed from November 2020 to Jun 2021.

All patients were already diagnosed as diabetic patients with type 2 diabetes mellitus according to diagnostic criteria.[(13)]

Also 150 healthy overweight male control aged 30-50 years were enrolled.

2.1.1 Patients Criteria

2.1.1.1 Inclusion Criteria

Male patients with T2DM were receiving 1500 mg /day metformin (500mg/8 hr.) from 6 months up to 3 years with no other diseases.

2.1.1.2 Exclusion Criteria

1. Subjects were receiving insulin and insulin secretagogues therapy or any other drug that interact with the OCT1 receptor, such as, diltiazem, verapamil, proton pump inhibitors, spironolactone, ketoconazole, or clopidogrel.
2. Patients with hyperthyroidism, hypothyroidism, serious renal failure, chronic hepatic diseases, malignant diseases and autoimmune diseases are excluded.

2.1.2 Ethical and Scientific Approval

- The scientific and ethical committee at the college of pharmacy – Kerbala University discussed and approved the proposal of the research.
- All participants were enrolled in the study after signing a written consent form that included a detailed explanation of the study's purpose and a request to complete a specially designed questionnaire.

2.1.3 Study Design

It is a cross-sectional study, with a total of 200 Iraqi males with type 2 diabetes mellitus disease were included and 150 apparently healthy males without any disease were served as a control group. From overnight fasted participants who are already taken metformin blood sample was collected for biochemical, hormonal, and genetic study.

All precautions have been taken in clinical settings to prevent infection of covid 19

2.2 Materials

2.2.1 Instruments and their Origin

All instruments used in this study are listed in table (2-1) accompanied by their manufacturing company

Table (2-1) Instruments and the manufacturing companies

Instrument	Company	Country
BS240Pro	Mindary	China
Centrifuge	Hettich	Germany
CL900 I	Mindary	China
Digital camera	Canon	England
Distillatory	GEL	Germany
Hood	Lab Tech	Korea
Incubator	Binder	Germany
Micropipettes	Slamed	Japan
Nano Pac 500 power supplier for electrophoresis	Cleaver	UK
PCR machine(Thermocycler)	Verity	United state
Sensitive balance	AND	Taiwan
UV-trans illuminator	Syngene	England
Vortex mixer	Human twist	Germany

2.2.2 Chemicals, Kits and their Suppliers

All chemicals and kits used in this study with their producing company are listed in the table (2-2)

Table (2-2) Chemicals and kits and their producing companies.

	Chemicals and Kits	Company	Country
Chemicals	Agarose	Bio Basic	Canada
	Ethanol	Hayman Kimia	UK
	Ethedium Bromide	Intron	Korea
	Nuclease free water	Bioneer	Korea
	TBE buffer	Bioneer	Korea
Biochemical Kits	Cholesterol Kit	Mindray	China
	Fasting serum glucose kit	Roche	Germany
	Glycosylated Hemoglobin kit	Minday	China
	HDL kit	Mindray	China
	Insulin kit	Mindray	China
	LDL kit	BioBase	China
	Omentin Kit	BioassayTecnology Laboratory	China
	Triglyceride kit	Mindray	China
Kits For Genetic Study	DNA extraction kit	Intron	Korea
	DNA ladder marker	Intron	Korea
	PCR PreMix Kit	Bioneer	Korea
	Primers OCT 1 rs 72552763 ATGAT>AT	Bioneer	Korea

2.3 Methods

2.3.1 Samples Collections

After overnight fasting, blood samples were collected from all patients and healthy controls, and the blood samples was divided into two parts, first, part (2ml) was kept in an EDTA tube for HbA1c test and DNA extraction and the second part (3ml) was kept in gel tube for serum isolation for hormonal and biochemical tests.

2.3.2 Biochemical Assay Methods

2.3.2.1 Determination of Glycemic Indices

2.3.2.1.A Estimation of Fasting Serum Glucose

The principle of fasting serum glucose (FSG) estimation is; Hexokinase enzyme catalyzes the phosphorylation of glucose to glucose -6-phosphate by ATP and glucose level is estimated by UV, in the presence of NADP, glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to gluconate -6-phosphate. There is no other carbohydrate that is oxidized. The rate of NADPH formation during the reaction is photometrically measured and is directly proportional to the glucose concentration.[113]

2.3.2.1.B Estimation of Fasting Serum Insulin

The CL-series insulin assay is a two-site immunoenzymatic assay that measures insulin levels. In the first step, a reaction cuvette is filled with sample, paramagnetic microparticles coated with monoclonal anti-insulin antibody, and monoclonal anti-insulin antibody-alkaline phosphatase

conjugate. Insulin in the sample binds to anti-insulin antibody-coated microparticles and anti-insulin antibody alkaline phosphatase-labeled conjugate to form a sandwich complex after incubation. The unbound substances are washed away, while microparticles are magnetically captured.

The substrate solution is added to the reaction cuvette in the second step, and the anti-insulin antibody-alkaline phosphatase conjugate in the immunocomplex retained on the microparticles catalyzes the reaction. The resulting chemiluminescent reaction is measured as a relative light unit by a photomultiplier built inside the system, the amount of insulin present in the sample is proportional to the relative light units generated during the reaction.(114)

2.3.2.1.C Estimation of Insulin Resistance and Beta cells function

Homeostasis model assessment (HOMA-IR) is a non-invasive, fast, cheap alternative, and reliable way to estimate insulin resistance, was developed by Matthews in 1985, it can estimate insulin resistance (IR) by using the homeostasis basal fasting concentrations of glucose and insulin.(27) (HOMA-IR) was calculated as.(115)

HOMA-IR = [fasting insulin (μU/ml) × fasting plasma glucose (mg/dl)]/ 405

Beta-cell secretory capacity is expressed as Homeostasis Model Assessment-Beta cells function(HOMA-B) [116] HOMA-B calculated from fasting glucose and insulin levels by using the following formula;[115]

HOMA-B = 360 × fasting insulin (μU/ml)/[fasting plasma glucose (mg/dl) – 63]

2.3.2.1.D Estimation of Glycosylated Hemoglobin

For Glycosylated Hemoglobin (HbA1c) estimation, the blood sample kept in the EDTA tube is automatically hemolyzed with the BS240Pro system. This method uses the detergent tetradecyltrimethylammonium bromide (TTAB) in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocyte). All glycated hemoglobin at beta-chain N terminal have antibody recognizable regions identical to that of HbA1c and measured by this assay.

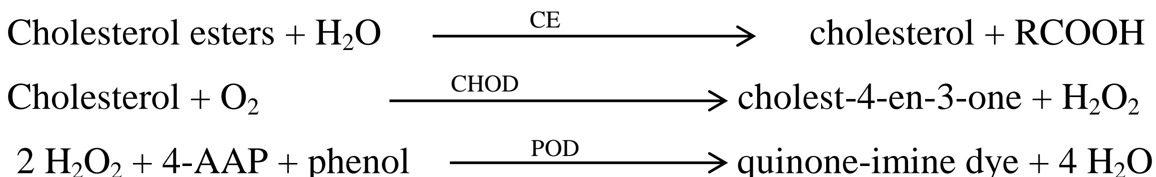
The reaction between glycohemoglobin and anti HbA1c antibody in the sample leads to form soluble antigen-antibody complex; this complex formation does not take place because the HbAq2c antibody site is present only once on the HbA1c molecule. The excess of anti- HbA1c antibody reacts with polyhapten and this leads to form an insoluble antibody-polyhapten complex which can be measured turbidimetric.[117]

Estimated average glucose (eAG) was derived from HbA1C level but presented in mg/dl instead of percentag and calculated based on the equation (eAG =28.7 × HbA1C – 46.7).[100]

2.3.2.2 Determination of Lipid Profile

2.3.2.2.A Estimation of Total Cholesterol

The enzymatic colorimetric method was used for the determination of total cholesterol (TC) in serum on BS240Pro in vitro. By the effect of the cholesterol esterase enzyme (CE), cholesterol esters are cleaved to free cholesterol and fatty acids. Then by cholesterol oxidase enzyme (CHOD) which oxidizes cholesterol to cholest -4-en-3-one and hydrogen peroxide .the hydrogen peroxide affects oxidative coupling of phenol and 4-aminoantipyrin (4-AAP) to form red quinone –imine dye by the effect of peroxidase enzyme (POD).

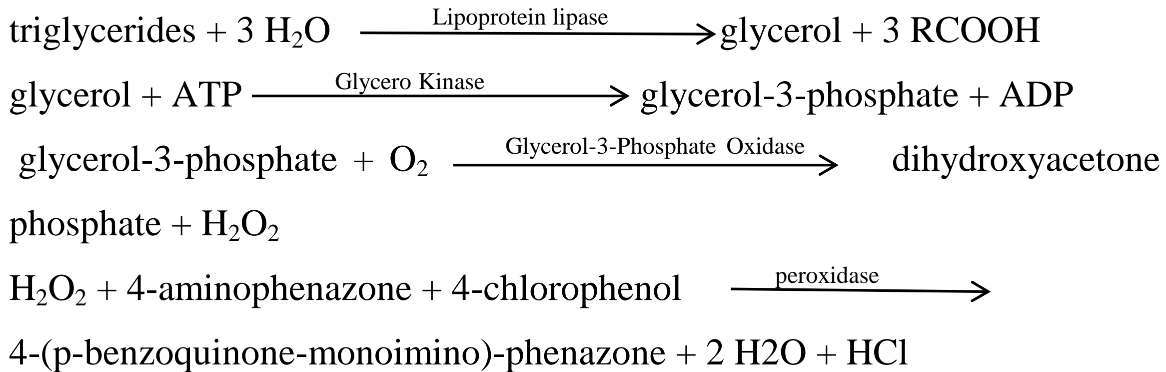


The cholesterol concentration is directly proportional to the color intensity of the dye formed and measured by the increase in absorbance at 512 nm.[118]

2.3.2.2.B Estimation of Serum Triglyceride

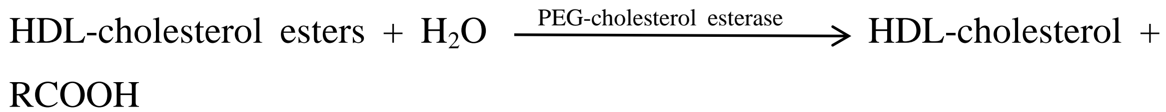
The enzymatic quantitative colorimetric method was used for the estimation of triglyceride (TG) in serum on the BS240Pro system in vitro. The serum triglyceride was measured enzymatically by using a series of coupled reactions in which triglycerides are hydrolyzed by lipase to glycerol. Glycerol then by using glycerol kinase and glycerol-3-phosphate oxidase oxidized to produce H2O2. H2O2 is quantitatively measured in a peroxidase

catalyzed reaction that produces a color, absorbance is measured at 500 nm. The color intensity is proportional to triglyceride level. [118]

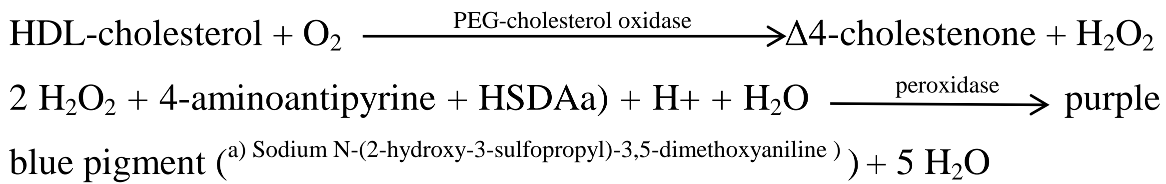


2.3.2.2.C Estimation of Serum High-Density Lipoprotein

On the BS240Pro system, an in vitro quantitative enzymatic colorimetric test was used to estimate high-density lipoprotein (HDL) in serum. Water-soluble complexes with low density lipoprotein(LDL), very low density lipoprotein (VLDL), and chylomicrons are formed in the presence of magnesium ions and dextran sulfate, and they are resistant to PEG-modified enzymes. Enzymatically determination of HDL by using enzymes cholesterase and cholesterol oxidase, together with PEG to the amino groups. Cholesterol esterase breaks down cholesterol esters quantitatively into free cholesterol and fatty acids. Cholesterol is oxidized by cholesterol oxidase to Δ4 cholestenone and hydrogen peroxide in the presence of oxygen.[119]



HDL-cholesterol esters + H_2O $\xrightarrow{\text{PEG-cholesterol esterase}}$ HDL-cholesterol + RCOOH



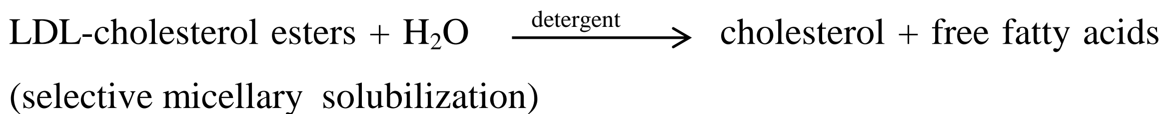
HDL-cholesterol + O_2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ Δ4-cholestenone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine + HSDAa) + H+ + H_2O $\xrightarrow{\text{peroxidase}}$ purple blue pigment (a) Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) + 5 H_2O

The amount of blue quinoneimine dye produced is proportional to the amount of HDL cholesterol present. The increase in absorbance at 583 nm is used to determine it.

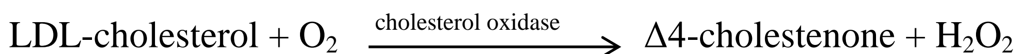
2.3.2.2.D Estimation of Serum Low-Density Lipoprotein

Cholesterol esters and free cholesterol in Low-Density Lipoprotein (LDL) are determined using a cholesterol enzymatic method that involves the use of cholesterol esterase and cholesterol oxidase in the presence of surfactants that selectively solubilize the only LDL. Surfactants and a sugar compound inhibit the enzyme reactions to lipoproteins other than LDL. Cholesterol levels in HDL, VLDL, and chylomicron are not recognized.



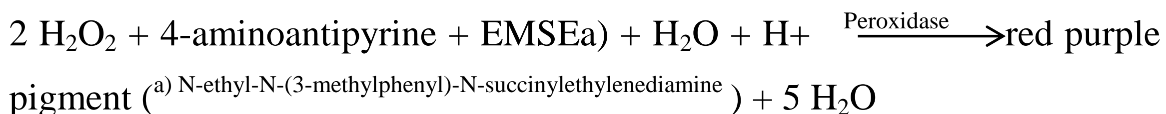
LDL-cholesterol esters + H_2O $\xrightarrow{\text{detergent}}$ cholesterol + free fatty acids (selective micellary solubilization)

Cholesterol esterase breaks down cholesterol esters quantitatively into free cholesterol and fatty acids.



LDL-cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ Δ4-cholestenone + H_2O_2

Cholesterol oxidase converts cholesterol to Δ4-cholestenone and hydrogen peroxide in the presence of oxygen.



2 H_2O_2 + 4-aminoantipyrine + EMSEa) + H_2O + H+ $\xrightarrow{\text{Peroxidase}}$ red purple pigment (a) N-ethyl-N-(3-methylphenyl)-N-succinylethylenediamine) + 5 H_2O

The hydrogen peroxide produced reacts with 4 aminoantipyrine and EMSE to form a red-purple dye in the presence of peroxidase. This dye's color intensity is proportional to cholesterol concentration and is measured using a photometer.[120]

2.3.2.3 Estimation of Serum Human Omentin-1

After the serum sample was collected and lifted to clotted for 10-20 minutes at room temperature and centrifuged at 2000-3000 round per minute (RPM) for 20 minutes, the enzyme-linked immunosorbent assay (ELISA) Kit was used. The plate has been pre-coated with a human omentin -1 antibody.Omentin-1 present in the sample was added and binds to antibodies coated on the walls .And then biotinylated human omentin -1 antibody is added and binds to omentin-1 in the sample. Then Streptavidin-HRP is added and binds to the biotinylated omentin-1 antibody. After incubation unbound streptavidin-HRP is washed away during a washing step. The substrate solution is then added and color develops in proportion to the amount of human omentin-1. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.[121]

2.3.2.4 Determination of Body Mass Index

The Body Mass Index (BMI) is a calculation based on a person's weight and height. The BMI is calculated by dividing the body weight by the square of the body height and is expressed in kilograms per square meter (kg/m2), with the mass in kilograms and the height in meters.[122]

$$\mathbf{BMI = Weight / (Height)^2}$$

The values fall in the range between 18.5-24.9 represent the normal weight, while between 25-30 represent overweight and above 30 is the obese one.

2.3.3 Genetic Analysis

2.3.3.1 Extraction of Genomic DNA from Blood Sample

G-spin Total DNA extraction kit from Intron was used to provide a fast and easy method for purification of total DNA from blood and various biological samples and to yield pure DNA suitable for storage and immediate application.

- Pipet 200 µl of whole blood and added into 1.5 microcenterfuge tubes.
- 20µl of proteinase K enzyme was added to the sample tube then mix by pulse vortex.
- 200µl of BL buffer was added into the sample tube and mixed thoroughly to yield a lysis solution.
- The mixture was left at room temperature for 2 minutes.
- The lysate was incubated at 56 ºc for 10 min with repeated mixing.
- The sample tube was centrifuged briefly to remove drops from the inside of the lid.
- 200µl of absolute ethanol was added into lysate and mix well by vortex and briefly centrifuge also.
- The mixture was applied carefully to the spin column, close the cap, and centrifuged at 13,000 rpm for 1 min, then the filtrate was discarded and a new collection tube is replaced.
- 700µl of WA buffer was added to the spin column and centrifuged for 1 min at 13,000 rpm and discard the filtrate.
- 700µl of WB buffer was added to the spin column and centrifuged for 1 min at 13,000 rpm and discard the filtrate then the collection tube

was replaced with another one for additional centrifuged for 1min to dry the membrane.

- The spin-column was replaced with a new 1.5 ml tube then 80 µl of buffer CE was added directly into to membrane and incubated for 1min at room temperature then centrifuged for 1min at 13,000 rpm to elute DNA.

2.3.3.2 Allele Specific Polymerase Chain reaction

2.3.3.2. A Primer Preparation

Polymerase Chain Reaction (PCR) was performed by using a specific primer to amplify OCT1 gene rs72552763. This primer was designed by using primer –BLAST software and purchased from Bioneer, Korea as a lyophilized product of different picomols concentrations. Each primer were dissolved in specific volumes of nuclease-free water to obtain a stock solution with a concentration of 100 pmol/µl after that, a diluted work solution was made by mixing 10 µl of each stock solution primer with 90µl of nuclease-free water. This work solution was kept at -20°C until use.

Table (2-3) Primer sequence of OCT1 rs72552763[123]

Primer	Sequence	Product Size (bp)
Un-delete	GCAGCCTGCCTCGTCATG	100
Del-ATG	GCAGCCTGCCTCGTCATT	100
O-R	AGTCACAACACTTTCCCCACA	-

2.3.3.2.B Optimization of Polymerase Chain Reaction Conditions

The PCR reaction was optimized, after several trials, to determine the best annealing temperature and the number of amplification cycles for the allele-specific PCR reaction .And the best concentration of both DNA and primer. Tables (2-4) and (2-5) show the components of PCR reactions for all amplified fragments and optimized PCR programs, respectively.

2.3.3.2.C Running the Polymerase Chain Reaction

The PCR reaction was performed out by combining PCR components with DNA solution and employing the optimized PCR programs as shown in Table (2-4)

Table (2-4) Mix reaction for genotyping of OCT1 rs72552763 in Polymerase Chain Reaction

Alleles: ATGAT>AT

Component	Volume (µl)
Forward primer	1.25
Reverse primer	1.25
DNA template	5
Deionized water	12.5
Premix	5

Table (2-5) Polymerase Chain Reaction conditions for genotyping of OCT1 gene (420del) rs72552763 Variant type: delin .Alleles ATGAT>AT

<table>
<tr><th>Steps</th><th>Temperature/c</th><th>Time/sec</th><th>Cycle</th></tr>
<tr><td>Denature template</td><td>95</td><td>4 minutes</td><td>1</td></tr>
<tr><td>Initial denaturation</td><td>95</td><td>40</td><td rowspan="3">35</td></tr>
<tr><td>Annealing</td><td>58</td><td>30</td></tr>
<tr><td>Extension</td><td>72</td><td>30</td></tr>
<tr><td>Final extension</td><td>72</td><td>5 minutes</td><td>1</td></tr>
</table>

2.3.3.3 Agarose Gel Electrophoresis

- Agarose gel was prepared by dissolving 1.5g of agarose powder in 100ml of 1x TBE buffer (PH 8).
- Heating the solution on a hot plate until boiling.
- Waiting for cooling then 2 µl of ethidium bromide was added to the solution.
- The comb was fixed at the end of the tray for making wells used for PCR product loading.
- The agarose was gently poured into the tray and waited to solidify at room temperature for 30 min.
- The comb was gently removed from the tray.
- The tray was fixed in an electrophoresis chamber and filled with a TBE buffer.
- PCR products were loaded into the wells directly.
- The electrophoresis apparatus's voltage was set to ensure an electrical field of 5 v.cm-1 for the distance between the cathode and anode.
- At the end of the run which is about 90 min, and ultraviolet trans-illuminator was used at 320-336 nm for band detection.
- The gel was photographed using a digital camera.

2.4 Statistical Analysis

The data of participants in this study were converted into a computerized database, revised for errors or inconsistencies, and then managed, processed, and analyzed by using the statistical package for social sciences (SPSS) version 26, IBM, US.

Scale variables are presented in mean, standard deviation (SD), While descriptive statistics for nominal (categorical) variables represented as frequency (number of participants) and proportion (percentage). Scales variables like Age and BMI that follow the statistical normal distribution, so parametric test was applied.

The Student's test was used to compare the means of two independent samples. To compare more than two means, one-way analysis of variances (one-way ANOVA) was used. The association between categorical variables was measured using the Chi square. Fisher's exact test was used as an alternative when the chi square was inapplicable.

For assessment of response to treatment, the odd ratio (OR) was calculated using logistic analysis of the SNP and 95% confidence intervals were calculated to estimate the significance of the OR.

Bivariate correlation test was used to estimate the association between Snip and mean of diabetic variables. Correlation coefficient (R) was calculated. The value of (R) ranged from 0 to 1, with the higher value indicating a stronger correlation (positive correlation). The sign of (R) indicated the direction of correlation, with a negative signed R value

indicating an inverse correlation and a positive (unsigned) R value indicating a direct correlation.

To be considered a significant difference or association, the level of significance was set at ($P \leq 0.05$). Finally, the findings and results were presented in tables and/or figures, each with an explanatory paragraph.

Chapter Three

Results

3. Results

3.1 Socio-demographic Data for Control and Type 2 Diabetic Patients

The socio-demographic data for 200 diabetic patients and 150 healthy men (control group) are demonstrated in the table (3-1). The age range was between 30-50 years with a mean ±SD of 40.89 ± 4.99 for control and 43.99 ± 5.59 for patients, there were significant statistical differences between diabetic patients and the control group regarding age. BMI was 29.07 ± 3.18 for control and 30.03 ± 3.46 for patients, this result showed that the BMI of T2DM patients was significantly higher than those of the healthy control group.

Concerning lifestyle, about (74.7%) have an active lifestyle in the control group and about (25.3%) with sedentary style while in the diabetic group about (62.5%) with an active lifestyle and (37.5%) with sedentary style, this result also shows significant differences between both group. Regarding the occupation, smoking, family history, and alcohol intake there were no significant differences between the study groups.

Table (3-1): Socio-demographic data of type2 diabetic patients and control group.

Variables		Groups		p-value
		Control (n=150)	Diabetics (n=200)	
Age (y)		40.89 ± 4.99	43.99 ± 5.59	**< 0.001 [S]**
BMI(kg/m^2)		29.07 ± 3.18	30.03 ± 3.46	**0.008 [S]**
Life Style	**Active**	112 (74.7%)	125 (62.5%)	**0.021 [S]**
	Sedentary	38 (25.3%)	75 (37.5%)	
Occupation	**Free**	59 (39.3%)	79 (39.5%)	0.318 [NS]
	Employee	91 (60.6%)	121 (60.5%)	
Smoking	**Yes**	53 (35.3%)	86 (43%)	0.185 [NS]
	No	96 (64%)	114 (57%)	
Family history	**Mother**	26 (17.3%)	48 (24%)	0.378 [NS]
	Father	27 (18%)	32 (16%)	
	Both	20 (13.3%)	38 (19%)	
	Non	77 (51.3%)	82 (41%)	
Alcohol	**Yes**	3 (2%)	4 (2%)	0.723 [NS]
	No	147 (98%)	196 (98%)	

Results are presented as mean ± SD, n= number of subjects and% percentage , p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant

3.2 Assessment of Metabolic Parameters for Control Group and Type2 Diabetes Mellitus Group

3.2.1 Glycemic Parameters of Healthy Control Group and Type2 Diabetic patients Group.

Data in the table (3-2) showed significant differences in all glycemic parameters (fasting blood glucose (FBG), glycated hemoglobin (HbA1c), fasting serum insulin (FSI), HOMA-IR, HOMA-B, and eAG level) between study groups, the glycemic parameters in diabetic patients was significantly higher than the healthy group,While HOMA-B is lower significantly in diabetic patients compared to the healthy group.

Table (3-2): Glycemic parameters of the healthy control group and type2 diabetic group.

Variables	Groups		p-value
	Control (n=150) mean ± SD	Diabetics (n=200) mean ± SD	
FBG (mg/dl)	98.98 ± 10.52	149.01 ± 27.65	**< 0.001 [S]**
HbA1c %	5.23 ± 0.41	7.47 ± 1.49	**< 0.001 [S]**
FSI (µu/ml)	13.58 ± 4.56	16.32 ± 5.39	**< 0.001 [S]**
HOMA-IR	3.38 ± 1.35	6.09 ± 2.43	**< 0.001 [S]**
HOMA-B	140.61 ± 42.77	74.34 ± 33.89	**< 0.001 [S]**
eAG (mg/dl)	103.49 ± 11.64	167.59 ± 38.46	**<0.001 [S]**

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant

3.2.2 Lipid Profile of Healthy Control Group and type2 Diabetic Patients Group

The data in the table (3-3) showed significant differences in TG level which was significantly higher in the diabetic group, while LDL and HDL level was significantly lower in type2 DM compared to the control group. TC level showed no significant differences between the two groups.

Table (3-3): Lipid profile parameters in the healthy control group and type2 DM group

Variables	Groups		p-value
	Control (n=150) mean ± SD	**Diabetics (n=200) mean ± SD**	
TC (mg/dl)	176.93 ± 41.18	174.19 ± 37.68	0.517 [NS]
TG (mg/dl)	147.52 ± 42.48	159.58 ± 40.97	**0.008 [S]**
LDL(mg/dl)	109.27 ± 30.41	102.43 ± 28.61	**< 0.041 [S]**
HDL(mg/dl)	41.86 ± 13.69	35.85 ± 9.91	**< 0.001[S]**

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant

3.3 Genetic Analysis

Analysis was conducted to assess the association between the OCT1 polymorphism 420del (rs72552763) with the pathogenicity of type2 DM.

- The 420del (rs72552763) ATG-ATG (wild allele), ATG-Del (heterozygous type), and Del-Del (mutant type), as shown in figure (3-1). Results in the table (3-4) showed that the frequencies of the alleles of OCT1 were not significantly different between the control group and type2 DM group (p>0.05). The diabetic patients had 38.5% of wild allele and 36% of heterozygous allele and 25.5% of the mutant allele, while the control group had 35.3% of wild allele and 34.7% of heterozygous allele, and 30% of the mutant allele.

Figure (3-1) Polymerase chain reaction amplification of OCT1 gene. Delin. Alleles: ATG>AT showing the ATG and Del alleles in size 100bp.

Table (3-4): Alleles frequencies of 420del in control and diabetic patients.

Alleles	Groups		p-value
	Control (n=150) n(%)	Diabetics (n=200) n(%)	
ATG-ATG	53 (35.3%)	77 (38.5%)	0.652 [NS]
ATG-Del	52 (34.7%)	72 (36%)	
Del-Del	45 (30%)	51 (25.5%)	
Results are presented as numbers and percentages, p<0.05 considered significantly different, [S]= Significant, [NS]= Non-significant			

According to logistic regression analysis, the data in the table (3-5) indicate there was no significant association between different alleles with the pathogenesis of type 2 diabetes mellitus.

Table (3-5): Logistic regression analysis of 420del to predict type2 diabetes mellitus

Variables	OR (95% CI)	p-value
ATG-ATG	1[a]	-
ATG-Del	0.953 (0.578-1.571)	0.850 [NS]
Del-Del	0.780 (0.458-1.328)	0.360 [NS]
Results are presented as numbers, p<0.05 considered significantly different, [S]; Significant, [NS]; Non-significant, OR: Odds ratio, CI; Confidence interval, a; reference category		

3.4 Effect of Organic Cation Transporter 1 Polymorphism 420del (rs72552763) on the Metabolic Response to Metformin.

3.4.1 Effect of 420del (rs72552763) on the Glycemic Profile of Diabetic Patients in Response to Metformin.

The results in a table (3-6) showed the level of (FBG, HbA1c, eAG) was significantly higher in mutant allele than in wild allele and in mutant allele than heterozygous allele, while there were no significant differences between wild allele and heterozygous allele. The results also showed the levels of (FSI, HOMA-IR, BMI) was significantly higher in mutant allele than in wild allele, while there were no significant differences between wild allele and heterozygous allele and between heterozygous allele and mutant allele. HOMA-B level showed no significant differences between all alleles.

Table (3-6): Glycemic parameters in the diabetic patients according to 420del.

Glycemic parameters	*OCT1 Alleles*			*P-value*
	***ATG-ATG* mean ±SD**	***ATG-Del* mean ± SD**	***Del-Del* mean ± SD**	
FBG (mg/dl)	142.92 ± 24.98	146.72 ± 28.82	161.43 ± 26.3	**0.001 [S]** [b, c]
HbA1c%	6.98 ± 1.15	7.33 ± 1.33	8.39 ± 1.14	**< 0.001 [S]** [b, c]
FSI (µu/ml)	14.89 ± 5.1	16.36 ± 5.56	18.44 ± 4.96	**0.001 [S]** [b]
HOMA-IR	5.35 ± 2.23	6.02 ± 2.41	7.38 ± 2.25	**< 0.001 [S]** [b]
HOMA-B	71.55 ± 27.94	72.87 ± 26.03	72.16 ± 29.63	0.367 [NS]
eAG (mg/dl)	153.72 ± 32.96	163.56 ± 31.24	194.22 ± 32.59	**< 0.001 [S]** [b, c]
BMI (kg/m^2)	28.97 ± 2.76	30.23 ± 3.61	31.35 ± 3.76	**< 0.001 [S]** [b]

Results are presented as mean ± SD, p<0.05 considered significantly different,
[S]= Significant, [NS]= Non significant
a; ATG-ATG vs. ATG-Del, b; ATG-ATG vs. Del-Del c; ATG-Del vs. Del-Del

As illustrated in the table (3-7) The logistic analysis of the 420del (rs72552763) of the diabetic patients according to glycemic control showed that the significant decrease in the level of HbA1c and HOMA-1R were in wild allele (ATG-ATG) than those in mutant allele (Del-Del) which indicate that the response to metformin regarding HbA1c and HOMA-IR level was significantly related to the wild allele than mutant allele of OCT1 SNP by 2.028 and 1.454 respectively ($p<0.001$). While there were no significant

differences between wild allele and heterozygous allele regarding the response to metformin in both parameters.

Table (3-7): Logistic regression analysis of 420del with HbA1c and HOMA-IR of the diabetic patients.

Parameters	OCT1 alleles	OR (95% CI)	p-value
HbA1c	ATG-ATG	1[a]	-
	ATG-Del	1.22 (0.953-1.563)	0.122 [NS]
	Del-Del	2.028 (1.521-2.704)	**<0.001[S]**
HOMA-IR	ATG-ATG	1[a]	**-**
	ATG-Del	1.131 (0.982-1.304)	0.88 [NS]
	Del-Del	1.454 (1.233-1.715)	**<0.001[S]**

Results are presented as numbers, p<0.05 considered significantly different, [S]; Significant, [NS]; Non-significant, OR: Odds ratio, CI; Confidence interval, a; reference category

The results in table (3-8) showed a significant positive correlation of the 240del with FBG, HbA1c, FSI, HOMA-IR, eAG level. (p<0.001) and non-significant correlation with HOMA-B (P=0.785)

Table (3-8): Correlation of the 420del with glycemic parameters of diabetic patients.

OCT1 snp vs. Diabetic Parameters	*Correlation coefficient (R)*	*P- value*
FBG (mg/dl)	0.253	**<0.001 [S]**
HbA1c %	0.361	**<0.001 [S]**
FSI (µu/ml)	0.257	**<0.001 [S]**
HOMA-IR	0.323	**<0.001 [S]**
HOMA-B	0.019	0.785 [NS]
eAG mg/dl	0.361	**<0.001 [S]**
p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant		

The effect of the genotype distribution of 420del in diabetic patients according to the level of HbA1c (cut-off point 6.5%)[13] showed no significant differences (p>0.05) in the distribution of 420del between adequate and inadequate groups of diabetic patients as illustrated in the table (3-9).

Table (3-9): Effect of the genotype distribution of 420del OCT1 in diabetic patients on glycemic control according to the level of HbA1c

OCT1 alleles	HbA1c %			p-value
	Total	Adequate n(%)	Inadequate n(%)	
ATG-ATG	77	29 (37.7%)	48 (62.3%)	0.085 [NS]
ATG-Del	72	24 (33.3%)	48 (66.7%)	
Del-Del	51	10 (19.6%)	41 (80.4%)	

Results are presented as numbers and percentage, p<0.05 considered significantly different,[S]= Significant, [NS]= Non significant, cut-off point of HbA1c = 6.5%

While there was a significant difference ($p<0.05$) in the effect of the genotype distribution of OCT1 in diabetic patients according to the level of HOMA-IR (cut of point =3.377) between both groups (adequate and inadequate) as illustrated in the table (3-10). In which (28.6%) from the patient with wild allele had adequate control and (20.8%) with heterozygous allele and only (5.9%) in mutant allele had adequate control

Table (3-10): Effect of the genotype distribution of 420del OCT1 in diabetic patients on glycemic control according to the level of HOMA-IR

OCT1 alleles	HOMA-IR			p-value
	Total	Adequate n(%)	Inadequate n(%)	
ATG-ATG	77	22 (28.6%)	55 (71.4%)	**0.007 [S]**
ATG-Del	72	15 (20.8%)	57 (79.2%)	
Del-Del	51	3 (5.9%)	48 (94.1%)	

Results are presented as numbers and percentages, $p<0.05$ considered significantly different, [S]= Significant, [NS]= Non-significant, mean HOMA-IR of control group = 3.377 used as a cut-off point

3.4.2 Effect of 420del (rs72552763) on Lipid Profile of Diabetic Patients in Response to Metformin.

The lipid profile of the diabetic patients according to 420del was shown in table (3-11), the results revealed that the levels of TG and LDL were significantly higher in heterozygous allele than in wild allele and in mutant allele than wild allele while there were no significant differences between mutant allele and heterozygous allele. The results showed also there were no significant differences in levels of TC and HDL between different alleles of 420del.

Table (3-11): lipid profile of the diabetic patients according to 420del.

Lipid profile parameters	*OCT1 alleles*			*P-value*
	ATG-ATG mean± SD	*ATG-Del mean± SD*	*Del-Del mean± SD*	
TC(mg/dl)	170.55 ± 35.23	179.31 ± 40.36	172.45 ± 37.26	0.343 [NS]
TG (mg/dl)	141.83 ± 40.61	168.51 ± 39.66	173.76 ± 33.68	**< 0.001 [S][a, b]**
LDL (mg/dl)	92.21 ± 22.91	107.79 ± 31.79	110.31 ± 27.55	**< 0.001 [S][a, b]**
HDL (mg/dl)	36.9 ± 9.91	36.49 ± 9.2	37.35 ± 8.4	0.113 [NS]

Results are presented as mean ± SD, p<0.05 considered significantly different,
[S]= Significant, [NS]= Non-significant
a; ATG-ATG vs. ATG-Del, b; ATG-ATG vs. Del-Del c; ATG-Del vs. Del-Del

3.5 Omentin-1 Concentration in Healthy Control Group and Diabetic Patients group

The data in the table (3-12) showed no significant differences ($p>0.05$) in omentin-1 concentration between the healthy control group and type2 DM group.

Table (3-12): Omentin-1 concentration in healthy control group and diabetic patients group.

Variable	Groups		p-value
	Control (n=150) mean± SD	Diabetics (n=200) mean± SD	
Omentin (ng/ml)	243.21 ± 50.81	238.83 ± 46.59	0.404 [NS]

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant

3.5.1 Effect of Organic Cation Transporter 1 Polymorphism 420del (rs72552763) on omentin-1 concentration in diabetic patients.

The data in the table (3-13) showed that there was no significant difference in the level of omentin-1 concentration with different alleles of 420del OCT1 snip ($p>0.05$)

Table (3-13) Omentin-1 concentration in diabetic patients in relation to 420del OCT1

Parameters	***OCT1 alleles***			***P-value***
	ATG-ATG	***ATG-Del***	***Del-Del***	
Omentin-1 ng/ml	240.9 ± 43.8	240.48 ± 49.01	233.38 ± 47.67	0.627 [NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

3.6 Blood groups Distribution in Healthy control group and Diabetic patients group

The data in the table (3-14) showed significant differences ($p<0.05$) in blood groups distribution between the healthy control group and patients group. Blood group type O had a high percentage of distribution in diabetic patients with 33% followed by blood group type A with 30% then group B with 25.5% and finally group AB with 7.5%.

Table (3-14) Blood groups distribution in the control group and diabetic patients.

Variables		Groups		p-value
		Control (n=150) n(%)	Diabetics (n=200) n(%)	
Blood groups	O	35 (23.3%)	66 (33%)	0.01 [S]
	A	49 (32.7%)	60 (30%)	
	B	39 (26%)	59 (25.5%)	
	AB	27 (18%)	15 (7.5%)	

Results are presented as numbers and percentage, $p<0.05$ considered significantly different, [S]= Significant, [NS]= Non significant

3.6.1 Association of Organic Cation Transporter 1 Polymorphism 420del (rs72552763) with ABO blood groups in diabetic patients

The analysis of the distribution of the 420del allele in association with ABO blood groups (A, B, AB, O) revealed that there was a significant difference in the distribution of different alleles (ATG-ATG, ATG-Del, and Del-Del) related to the number of diabetic patients in each blood group ($p<0.05$). The result showed that the higher percentage of the heterozygous allele was present in patients with group types O and A with 42.4%, 43.3% respectively, while patients with group B type had the higher percentage of wild allele with 47.5% and the patients with group type AB had the higher percentage of the mutant allele with 53.3% as shown in table (3-15)

Table (3-15): Distribution of420del alleles in each blood group.

Blood groups	*OCT1 Alleles*			*P-value*
	ATG-ATG n=77	*ATG-Del n=72*	*Del-Del n=51*	
O (n=66)	27 (40.9%)	28 (42.4%)	11 (16.7%)	**0.023 [S]**
A (n=60)	17 (28.3%)	26 (43.3%)	17 (28.3%)	
B (n=59)	28 (47.5%)	16 (27.1%)	15 (25.4%)	
AB (n=15)	5 (33.3%)	2 (13.3%)	8 (53.3%)	
Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non- significant				

Table (3-16) and figure (3-2) showed significant differences in the distribution of blood group of diabetic patients according to the types of alleles of 420del (p=0.023). A higher percentage of the mutant allele was presented in the patients with blood group type A, while the higher percentage of the wild allele was presented in patients with blood groups B and O.

Table (3-16) Distribution of blood groups in diabetic patients according to the types of alleles of 420del snip

OCT1 alleles	*Blood groups*				*P-value*
	O	***A***	***B***	***AB***	
ATG-ATG n=77	27 (35.10%)	17 (22.10%)	28 (36.40%)	5 (6.50%)	***P-value*** **0.023 [S]**
ATG-Del n=72	28 (38.90%)	26 (36.10%)	16 (22.20%)	2 (2.80%)	
Del-Del n=51	11 (21.60%)	17 (33.30%)	15 (29.40%)	8 (15.70%)	

Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non- significant

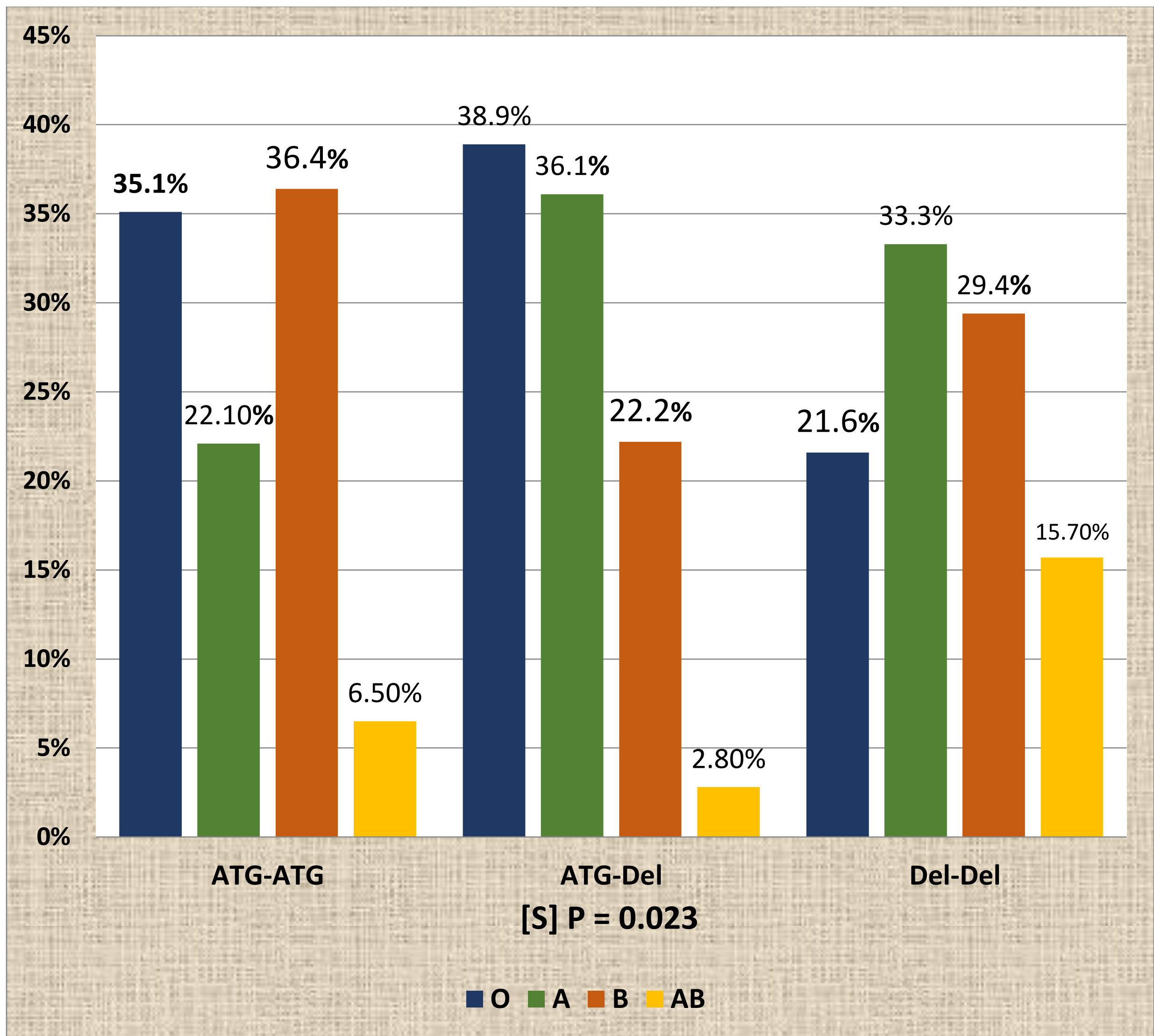


Figure (3-2): Distribution of blood groups in diabetic patients according to the types of alleles of 420de OCT1 snip

Chapter Four

Discussion

4.1 Socio-Demographic Data

Diabetes is one of the most serious health problems in the world and the most common metabolic disease in humans, characterized by abnormal glucose and fat metabolism. [124] The pathological abnormalities in T2DM are impaired insulin secretion from pancreatic beta-cell and/or impaired insulin action due to insulin resistance. The environmental and genetic consider important risk factors of T2DM.[61]

Table (3-1) demonstrated the demographic data of 200 patients with T2DM and 150 healthy men (control group), the age range between 30-50 years with a mean of 40.89 ± 4.99 for control and 43.99 ± 5.59 for patients, there were significant statistical differences between diabetic patients and the control group regarding age. BMI was 29.07 ± 3.18 for control and 30.03 ± 3.46 for patients, this result showed that the BMI of T2DM patients was significantly higher than those of the healthy control group given that obesity is a risk factor for insulin resistance development. [29,54]

Concerning lifestyle, the results showed a significant difference between both groups, about (74.7%) have an active lifestyle in the control group and about (25.3%) with sedentary style while in the diabetic group about (62.5%) with an active lifestyle and (37.5%) with sedentary style, sedentary behavior and high calories of an unhealthy diet are two major environmental factors that contribute to obesity, insulin resistance, and T2DM. In urbanized societies, sedentary behavior is more common, but with 30 minutes of moderate exercise per day and healthy eating, the risk of insulin resistance could be reduced. [125]

4.2 Metabolic Parameters Variation between Control Group and Type2 Diabetes Mellitus Group

The data in the table (3-2) showed a significant difference in all glycemic parameters including FBG, HbA1c, FSI, HOMA-IR, HOMA-B, and eAG levels between study groups, the glycemic parameters in diabetic patients was significantly higher than the healthy group, except HOMA-B was lower significantly in diabetic patients compared to the healthy group. Our results were compatible with Gandhi. et al. 2017 in which the routinely measured parameters like FBS, HbA1c, and FSI along with calculated parameters like HOMA-IR and eAG level were elevated in patients with T2DM(126). The increase in BMI and insulin resistance are the main causes of higher glycemic parameters, which contribute to increased glucose production in the liver and decreased glucose uptake in muscle and adipose tissue.(17) In response to insulin resistance, beta-cell hyperplasia and hyperinsulinemia occur as a compensatory mechanism to overcome the hyperglycemic situation of the disease.(127) The decreased insulin secretion (HOMA-B) and increased insulin resistance (HOMA-IR) were significantly associated with the development of T2DM.(12)

The results of lipid profile in the table (3-3) showed a significant difference in TG level, which was significantly higher in the diabetic group, while LDL and HDL level was significantly lower in T2DM compared to the control group. As determined by previous studies, TG levels were also significantly elevated and linked to type 2 diabetic subjects. And low HDL levels are a common abnormality in patients with T2DM.(65)

A significant increase in TG, as well as a decrease in HDL, was seen in diabetic patients with poor glycaemic control in an earlier study that reported significant correlations between HbA1c and lipid profiles, implying the importance of proper diabetes management in controlling dyslipidemia.[24] However, in our study we found a significant reduction in LDL level between the healthy control group and patients group which may be due to the effect of long term metformin therapy in diabetic patients, there were multiple mechanisms for metformin to reduce the LDL level, through reducing the lipolysis rate by increasing insulin sensitivity, slowing the conversion of free fatty acid to lipoprotein precursor in the liver, and by lowering the fraction of irreversibly glycated LDL which is removed less efficiently from the body.[128]

Diabetic patients with poor glucose control, dyslipidemia, and hyperlipidemia are common. Although hyperglycemia and dyslipidemia are independent significant risk factors for vascular complications, their interaction increases the risk of macro and microvascular complications. In patients with T2DM, dyslipidemia, which includes both quantitative and qualitative abnormalities of lipoproteins, can play a role in the pathogenesis of vascular complications.[129] Insulin resistance had a well-known effect on lipid metabolism. Insulin's ability to suppress lipolysis is affected by IR, which increases free fatty acid mobilization, reducing insulin's ability to inhibit hepatic lipoprotein synthesis.[130]

4.3 The Association of Organic Cation Transporter 1 Polymorphism with the Incidence of Type 2 Diabetes Mellitus

In the past decade, a total of 243 genetic loci have been reported in an attempt to investigate the underlying genetic pathogenesis of T2DM. However, as with most polygenic diseases, the discovered genes account for only about 20% of T2DM's inherited risk (heritability).(11) In this study analysis was conducted to assess the association of the OCT1 polymorphism 420del (rs72552763) with the pathogenicity of T2DM. The results in the table (3-4) showed that the frequencies of the alleles of OCT1 were not significantly different between the control group and the T2DM group, which indicated that there was no association between 420del (rs72552763) OCT1polymorphism and pathogenicity of T2DM.

According to logistic regression analysis, the data in the table (3-5) indicated that there was no significant association between different alleles with the pathogenesis of type 2 diabetes mellitus. Altall et al 2019 also tried to link between different types of SLC22A1 polymorphisms and the pathogenesis of T2DM, and they also found that the SLC22A1 gene polymorphisms rs628031 and rs461473, had no role in the pathogenesis of T2DM but may interact to affect the risk of T2DM in Saudi populations by altering glycemic parameters and other factors like BMI.(131)

4.4 Influence of Organic Cation Transporter 1 Polymorphism on Metformin Response in Patients with Type 2 Diabetes Mellitus.

Metformin, a biguanide derivative, is the first-line treatment for T2DM when combined with lifestyle changes. It enhances insulin sensitivity by increasing insulin receptor expression and increasing tyrosine kinase activity.[132] There is significant interindividual variability in metformin response; up to one-third of patients do not respond adequately, and both non-genetic and genetic factors influence the metformin effect. Different responses to pharmacological treatments may be explained by the presence of a genetic mutation or a combination of genotypes, so genetic variations associated with either pharmacological targets or drug metabolism are of particular interest. [123]

The majority of metformin pharmacogenetic studies have focused on the identification of gene variants associated with metformin pharmacokinetics.[7,123] Metformin enters hepatic cells to produce its effects as reduce hepatic glucose production by blocking gluconeogenesis and glycogenolysis through inhibiting respiratory-chain complex 1 in the mitochondria.[87,61] also cause impairing lipid synthesis and increasing insulin receptor expression, metformin requires membrane transport proteins encoded by solute carrier (SLC) genes for this purpose .[97]

Metformin has been well characterized as a substrate of OCTs, including SLC22A1 (OCT1), one of the most abundantly expressed transporters in the human liver and a key player in metformin uptake.[133]

In this study, the results in table (3-6) indicated that the glycemic parameters (FBG), (HbA1c), and (eAG) levels in diabetic patients treated with metformin were significantly higher in mutant allele than in wild and heterozygous allele. The results also showed that the levels of (FSI), HOMA-IR, and BMI were significantly higher in mutant allele than in wild allele, while there were no significant differences between wild allele and heterozygous allele and between heterozygous allele and mutant allele. HOMA-B level showed no significant differences between all alleles. The logistic analysis of the 420del (rs72552763) of the diabetic patients according to glycemic control table (3-7) showed the greater decrease in the level of HbA1c and HOMA-IR in wild allele compared to those in the mutant allele. The results showed that the patients with mutated alleles had significantly fewer responses to metformin, which could be due to reduced or lost OCT1 function, resulting in decreased hepatic metformin uptake which is an essential step for metformin action.

Our study was compatible with a study that has been performed in Tehran, Iran it was found that metformin's therapeutic effect is highly variable, depending on the genetic variation of OCT1.[(53)] Human OCT1 is highly polymorphic in ethnically diverse populations and many nonsynonymous polymorphisms of SLC22A1 are associated with a reduction in its activity. SLC22A1 genetic variants have been shown to modulate the pharmacokinetics of metformin after oral administration and reduce the therapeutic response, presumably by lowering the hepatic drug uptakes. [(104,133,134)]

The results of correlation between the glycaemic parameters and 420del SNP of OCT1 in a table (3-8) showed a significant positive correlation of the 240del with FBG, HbA1c, FIL, HOMA-IR, eAG levels which mean that the glycemic parameters of diabetic patients were elevated by the effect of 420del OCT1 SNP indicating a decrease in metformin action because of OCT1 polymorphism.

In the table (3-9), although the results showed no significant differences ($p>0.05$) in the distribution of alleles according to HbA1c cutoff value (6.5%) in terms of adequate and inadequate response, the mutant allele had a lower % of patients with the adequate response and the highest % with inadequate response to metformin due to defective transporter function and this indicated that this polymorphism affected HbA1c level.[100,135]

In the table (3-10) the results showed a significant difference ($p<0.05$) in the distribution of alleles according to the HOMA-IR (cut of point =3.377) in terms of adequate and inadequate response. The mutant allele had a lower % of patients with the adequate response (5.9%) and the highest % with inadequate response to metformin (94.1%) due to having a defective transporter function and this indicated that this polymorphism affected the HOMA-IR level.[85]

The effect of metformin on the lipid profile of the diabetic patients according to 420del was shown in table (3-11), the results revealed that the levels of TG and LDL were significantly higher in heterozygous and mutant allele than wild allele while there were no significant differences between mutant allele and heterozygous allele. Also, there were no significant differences in levels of TC and HDL between different alleles of 420del.

OCT1 dysfunction causes a reduction in metformin uptake by the liver, results in lower levels of metformin, and decreases its hypolipidemic activity by reducing the rate of lipolysis and fatty acid oxidation which is mediated by increasing the expression and activity of AMP-activated protein kinase and promoting the clearance of VLDL-triglycerides.[73,131] The results of previous studies were compatible with our results about the effect of SLC22A1 polymorphism on LDL level.[10,136]

4.5 Analysis of Omentin-1 Level in Diabetic Patients and its Association with 420del OCT1 Polymorphism

Omentin-1 may represent a biomarker of different metabolic diseases. It is an anti-inflammatory adipokine, predominantly expressed in stromal vascular cells of visceral adipose tissue and it promotes insulin signaling. The pathological mechanisms of vascular and metabolic disorders ,obesity, atherosclerosis, hypertension and cardiovascular disease are related to the circulating omentin-1 level. Macro- and micro-vascular complications of diabetes mellitus are also associated with it.[58-60]

In our study, the data in the table (3-12) showed no significant differences ($p>0.05$) in omentin-1 concentration between the healthy control group and the T2DM group. And regarding the effect of 420del (rs72552763) SNP on omentin-1 concentration in diabetic patients, the data in the table (3-13) showed that there was no significant difference in the level of omentin-1 concentration with different alleles of 420del OCT1 SNP ($p>0.05$). The possible explanation of these results was that the level of omentin-1 was affected by metformin treatment and many studies have suggested that metformin can reduce inflammation by modulating TNF-ά,

IL-6, IL-18, and IL-1 levels which protects omentin-1 gene expression from the negative effects of inflammatory burdens; .[137,138]

4.6 Analysis of Blood Groups Distribution in Diabetic Patients and its Association with 420del OCT1 Polymorphism

Blood groups are classified into types A, B, AB, and O in the ABO system, based on the presence or absence of inherited antigenic substances on the surface of red blood cells. Depending on the blood group system, these antigens can be proteins, carbohydrates, glycoproteins, and glycolipids.[139] Blood group frequencies differ between races and ethnic groups. [140]

Antigens in blood groups are genetically determined and play a key role in blood transfusion safety, hereditary patterns, and disease susceptibility.[141] Because of the biological and immunological roles of blood groups in diseases like gastric cancer, hypertension, and myocardial infarction, as well as the role of blood groups in metabolic diseases like obesity and diabetes, it was critical to examine them. [142,143] In immunology, both blood groups and diabetes have a genetic basis, and they can be mutually dependent. [120]

The pathophysiologic mechanisms that link ABO blood group phenotypes to T2DM are unknown. There are, however, a few possibilities: the first is that the ABO blood group has been linked to T2DM-related molecules, particularly A and B antigens, which have been linked to higher levels of plasma lipids and inflammatory markers like soluble intercellular

adhesion molecule 1 (ICAM-1), E-selectin, P-selectin, and tumor necrosis factor 2 (TNF-2), which are well-known inflammatory mediators for type 2 diabetes. Furthermore, inflammatory cytokines secreted by the endothelium in response to A and B antigen expression had an endocrine effect, causing insulin resistance in the liver, skeletal muscle, and vascular endothelial tissue, resulting in the clinical manifestation of T2DM. [144]

In this study, the data in the table (3-14) showed significant differences ($p<0.05$) in blood groups distribution between the healthy control group and patients group. Blood group O had a high percentage of distribution in diabetic patients followed by blood group A, group B, and finally, group AB. Our findings were consistent with a previous study conducted in Al Anbar, Iraq, which found that certain ABO systems are associated with an increased risk of type 2 diabetes, and that group O has a higher risk of developing diabetes than other blood groups O>A>B>AB. [145] The same findings were found in a study conducted in Baghdad, Iraq, which linked blood group O to an increased risk of diabetes, hypercholesterolemia, and hypertension due to lower levels of factor VIII and von Willebrand factor in the plasma of people with blood group O. Low levels of these factors raises the risk of arterial and venous complications.[146]

This study is the first study that evaluates the association of 420del (rs72552763) OCT 1 polymorphism with types of blood groups. The ABO blood group genes are mapped at 9q34.2 region, most blood antigens are the end product of a single gene, and genetic changes such as deletions, and inversions, insertions, alternative splicing, and single-nucleotide polymorphisms (SNPs) can result in antigenic changes, but can also result in the emergence of new antigens or even complete loss of expression. As a

result, the ABO blood group has been linked to many genetic disorders and can be found in a patient's genetic profile.[147] The ABO blood group alleles may have an impact on the systemic inflammatory state and immune response to certain cancers and diseases, according to a genome-wide association study. [148]

The analysis of the distribution of the 420del alleles in association with ABO blood groups revealed that there was a significant difference in the distribution of different alleles in each blood group ($p<0.05$). The patients with blood group B had the higher % of reference allele with 47.5% while the patients with group AB had the higher % of the mutant allele with 53.3% as shown in table (3-15). There were only 15 patients with blood group AB in our sample more than half of them had a mutant allele of the OCT1 SNP, indicating that diabetic patients with blood group AB had a higher chance of having a mutant allele of the OCT1 SNP than reference or heterozygous alleles, and thus had a lower response to metformin therapy in lowering blood glucose levels.

In Table (3-16) and figure (3-2) the results revealed that there were significant differences in the distribution of 420del alleles among different blood groups. Patients with blood group A had a higher percentage of the mutant allele, while patients with blood groups B and O had a higher percentage of the wild allele. The patients with blood group A had a lower response to metformin therapy duo to loss the function of OCT1 transporter, which results in therapeutic failure and uncontrolled DM.

4.7 Conclusions

These data provide a proof of concept, in setting that

1- The OCT1 polymorphism can be considered as one of the genetic factors responsible for heterogeneity in metformin response in male patients with T2DM in Kerbalaa. So our study shows evidence for a role of OCT1 polymorphism in the variability of metformin response in T2DM patients.

2- Patients with wild and heterozygous allele of OCT1 SNP showed different degrees of significant response to metformin treatment. While patients with a mutant allele of OCT1 SNP showed non-significant response to metformin treatment.

3- Polymorphism of OCT1 SNP is not associated with the incidence of T2DM.

5- Pharmacogenetic approaches like this could lead to new insights into the mechanisms affecting metabolic dysfunction, as well as new therapeutic targets for more precise interventions in type2 DM.

4.8 Recommendations and Future works

1- Further studies will be necessary to evaluate the impact of other genetic variants associated with metformin response like other OCTs and MATEs.

2- Studying other OCT1 SNPs to find out the effect of multiple OCT1 polymorphisms on response to metformin in patients with T2DM.

3- To proof the effect of transporter gene polymorphism on metformin bioavailability, the metformin plasma level should be determined.

4- Larger scale study contains more patients with T2DM and healthy control participants from different Iraqi cities are required.

5- In the clinical setting, We recommend that genetic tests should be developed to predict a person's response to metformin treatment and that personalized drugs with greater efficacy and safety should be developed.

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List of Normal Laboratory Value	
Parameters	**Normal Value**
TC	<200 mg/dl
FBS	<120 mg/dl
FSI	<25 µu/ml
HbA1c	6.5%
HDL	40< HDL >60
LDL	<100 mg/dl
TG	<150 mg/dl
Omentin-1	≥ 200 ng/ml

النتائج : أظهرت النتائج بأن تعدد الاشكال الجينية لناقل الكاتيون العضوي كان متشابها بين مرضى السكري والرجال الاصحاء, اما بالنسبة للأستجابة لعلاج المتفورمين لمرضى السكري والحاملين للأليل السائد والأليل المتنحي فقد أظهروا استجابة احصائية واضحة و بدرجات متفاوتة أما المرضى الحاملين للأليل الطافر لم يظهروا أستجابة علاجية للمتفورمين.

ألاستنتاج : أن الاختلاف الجيني لناقل الكاتيون العضوي قد يودي الى التفاوت في الاستجابة لعلاج المتفورمين لدى مرضى السكري, ولكنه غير مرتبط بالتسبب بالاصابة بمرض السكري من النوع الثاني

الخلاصة

الخلفية : مرض السكري هو واحد من أخطر المشاكل الصحية في العالم وأحد أمراض التمثيل الغذائي الأكثر شيوعا عند البشر, ويتميز بالتمثيل غير الطبيعي للسكر والدهون. الاسباب المرضية لدى مرض السكري من النوع الثاني هي اما ضعف إفراز الأنسولين من خلايا البنكرياس بيتا و / أو ضعف عمل الأنسولين بسبب مقاومة خلايا الجسم للأنسولين. تعتبر العوامل البيئية والجينية من اهم العوامل الخطرة والمسببة لمرض السكري وقد تم تحديد العديد من الجينات التي تساهم في زيادة الاصابة بمرض السكري عن طريق الدراسات المتعددة و المرتبطة بنطاق الجينات.

يعتبر المتفورمين الاختيار الاول لعلاج مرض السكري من النوع الثاني في العقود الاخيرة. حيث يعمل المتفورمين على انسجة الجسم المستهدفة للأنسولين مثل العضلات والكبد والانسجة الدهنية. تعتبر الاختلافات الجينية احد الاسباب المهمة والمؤدية الى التفاوت في الاستجابة لعلاج المتفورمين.

ناقل الكاتيون العضوي يلعب دور مهم في عملية ادخال المتفورمين الى الكبد والتي تعتبر الخطوة الاولى والاهم لعمل المتفورمين داخل الكبد, وجدت الدراسات الحديثة ان التعدد في الاشكال الجينية لهذا الناقل تؤثر بصورة مباشرة على الاستجابة لعلاج المتفورمين لدى مرضى السكري.

الهدف من الدراسة : الغرض من الدراسة هو للتحقق من وجود و تأثير تعدد الاشكال الجينية لناقل الكاتيون العضوي على التسبب بالإصابة بمرض السكري من النوع الثاني لدى الرجال في كربلاء, اضافة الى دراسة تأثير تلك الاشكال الجينية على الاستجابة لعلاج المتفورمين لدى الرجال المصابين بمرض السكري من النوع الثاني في العراق.

المرضى والطرق : هذه الدراسة المقطعية تمت بمشاركة 150رجل سليم و 200 رجل مصاب بمرض السكري من النوع الثاني ممن يتناولون دواء المتفورمين لعلاج السكري (بتركيز 1500 ملغ/اليوم) وتتراوح أعمارهم بين 30-50 سنة, تم إجراء الاختبارات الكيميائية الحيوية والدراسات الجينية لجميع والمشاركين في الدراسة لغرض تحديد تأثير التعدد الجيني للناقل الكاتيوني على الاستجابة لعلاج المتفورمين.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة

تأثير تعدد الشكل الجيني لناقل الكاتيون العضوي الاول على الاستجابة العلاجية للميتفورمين للرجال المصابين بمرض السكري من النوع الثاني في محافظة كربلاء

رسالة مقدمة الى

كلية الصيدلة في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الادوية و السموم

من قبل

هاله كريم جبار

(بكالوريوس صيدلة/ جامعة بغداد 2008)

بإشراف

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2021 ميلادي **1443 هجري**