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Association between Lipids and Thyroid Hormone Profiles with ABCA1 Gene Polymorphism for Type-II-Diabetes Mellitus Patients in AL-Muthanna Province

A Thesis

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By

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DIDECATION

This thesis is dedicated ……

To all Turkmen cities especially my dear city Tuzkhormatu ……..

To my honorary and supporting in all time ….

My father…

To who continually learns, grows and develops and who has been a source of encouragement and inspiration throughout my life,

My mum

To my life candlelight and operatives in the happiness moments

My brothers & sisters

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Abstract

Adenosine triphosphate-binding cassette transporter A1 (ABCA1) plays an important role in lipid metabolism and is involved in diabetes. The present study is undertaken to detect the possible association of V825I polymorphism in the ABCA1 gene with serum lipid profile among 100 Type -2- diabetes mellitus patients (46males, 54females) and 50 normal subjects(26males, 24females), collected between November 2014 to July 2015. Who attended to Al-Hussein hospital in AL-Muthanna province/Iraq. Serum parameters are also correlated with body mass index, sex and age. Each patient and normal subject are classified into three sub groups according to their Body Mass Index(BMI) (18.5-24.9 normal, 25-29.9 over weight and ≥30kg/m^2 obese), and also classified into three sub groups according to their age (40-49.9 G1, 50-59.9 G2, ≥60 year G3).

The chemical analysis which include(fasting blood sugar, total cholesterol, triglycerides and high density lipoprotein) by spectrophotometer technique, and the low density protein and very low density lipoprotein) it calculated by (Fired Wald equation). Either for hormonal testing which included (Thyroxin, Trithyropein and Thyroid Stimulating hormone) by using (Enzyme linked immune sorbent assay).

The results show that there is a highly significant increase ($P=\leq 0.05$) in level of Fasting Blood Sugar(FBS) for all classes of BMI, age and gender; A significant increase in the level of Total cholesterol, Triglycerides , Low density lipoprotein and Very low lipoprotein($P=\leq 0.05$) for class 2,1,2 and 1 of BMI respectively. Also significant increase in the level of TC, LDL and VLDL for class G1 of age only, and the level of TC for male only ($P<0.05$) in patients with T2DM compared with the normal group.

The present study detects that there is that there is a positive correlation between FBS with BMI(r=0.2390, p= 0.0463), TG(r=0. 1836,p= 0.1839), VLDL(r= 0.1836, p= 0.1839), and with thyroid hormones(T3(r= 0.1480,p= 0.4358), T4(r= 0.1039, p= 0.4544), TSH(r= 0.05943, p= 0.6694), while there is no correlation between FBS with each of TC(r= 0.04674, p= 0.7371), HDL (r= 0.03814, p= 0.7842), and LDL(r= 0.1236, p= 0.3731).

To studying the relation between Adenosine binding cassette transporter protein member 1(ABCA1) gene, used polymerase chain reaction (PCR) to amplification of gene which contain 525bp in ABCA1in locus V825I by using Taq 1 enzyme, the absence of cutting site refer to GG (525bp), while the presence and absence the cutting site in gene refer to GA (525bp, 302bp and 223bp) with remind the origin gene, either the presence the cutting site refer to AA (302bp and 223bp).

The frequency of GG genotype and G allele are higher in normal groups compared to patients (58% vs. 56% and 70% vs. 67%, respectively), converse to AA genotype (18% vs. 22%) and A allele (30% vs. 33%) higher in patients compared to normal groups. Data also show a significant relationship between ABCA1 gene polymorphism with each of TG and VLDL (P=0.007 for each). But, we could not find a relationship between ABCA1 polymorphism and another lipid concentrations.

Results indicate that the frequency of GG genotype for overweight and obese and frequency of AA for obese are significantly higher ($P<0.05$) with patients compared to normal groups, while there is no significant difference in frequency of GA with patients compared to normal groups. The results suggested that the frequency of GG genotype is significantly higher ($P\leq 0.005$) in patient individuals compared to normal according to the age of more than 50 year only. It explained the absence of significant differences of GA and AA gene polymorphism for all healthy and patients age groups.

Results found that there is no difference in GA and AA genotypic frequencies between males and females in the both normal and patient groups, except the highly significant ($P<0.05$) in frequency of GG genotype which is higher in female patients compared to normal subject.

Concluded there is no relationship between ABCA1 gene and lipid profile and also found G allele may be a protective factor against diabetes mellitus in Al-Muthanna population.

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Abbreviations	***Whole phrase***
A	Absorbance
ABC A1gene	ATP Binding Cassette Transportera1
ADA	American Diabetic Association
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
CHD	Coronary Heart Disease
DIT	Di-Iodotyrosine
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immune-Sorbent Assay
FBS	Fasting Blood Sugar
FFAs	Free Fatty Acids
FHA	Familial Hyperalphalipoproteinemia
FPG	Fasting Plasma Glucose
FSH	Follicle Stimulating Hormone
GDM	Gestational Diabetes Mellitus
GH	Growth Hormone
GPCR	G-Protein-Coupled Receptor
HbA1c	Glyceted Hemoglobin
HCG	Human Chorionic Gonadotrophic Hormone
HDL	High Density Lipoprotein
IDDM	Insulin-Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IDL	Intermediate Density Lipoproteins
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
LPL	Lipoprotein Lipase
LXR	Liver-X Receptor
MI	Myocardial Infection
MIT	Monoiodotyrosine

mRNA	Messenger Ribonucleic Acid
NCDs	Non Communicable Diseases
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
PPAR-g	Peroxisome Proliferators Activated Receptor-G
rT3	Reverse Triiodothyronine Hormone
RXR	Retinoid X Receptor
T1DM	Type 1diabetes Mellitus
T2DM	Type II Diabetes Mellitus
T3	Triiodothyronine Hormone
T4	Thyroxin Hormone
TBG	Thyroxin-Binding Globulin
TC	Total Cholesterol
TD	Tangier Disease
TG	Triglyceride
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid-Stimulating Hormone
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization
MCP1	Monocyta- Chemo Attrago Protein-1(Cytosine
SIPR	Sphino Siney Phosphate Receptor
SRB1	Scavenger- Recite Class B Type
LCAT	Lecithin Cholesterol Acyl- Transferase
CETP	Cholesterol Ester Transfer Protein
TICE	Trans-Intestinal Cholesterol- EHIO
ROC	Republic of China

1.1 Introduction

Diabetes is an accumulated glucose in blood (Buse *et al*., 2003) Diabetes is one of the four main non communicable diseases (NCDs) identified by the World Health Organization (WHO), besides the cardiovascular disease, cancer, and chronic respiratory diseases (Unwin, 2010). It is a group of metabolic disease characterized by hyperglycemia from defects in insulin secretion type 1 diabetes mellitus (T1DM) or insulin action type 2 diabetes mellitus (T2DM) or both (ADA, 2005; Emanuele *et al*., 2008). World Health organization (WHO) reported that there are (2.8%) in the world suffering from diabetes(Wild *et al*., 2004).The increasing number of individuals with type 2 diabetes indicates a global epidemic, prevalence of the disease was estimated to be 2.8% in 2000 and is predicted to increase to 4.4% by the year 2030.Although the prevalence of diabetes is expected to increase in all age groups, it has been estimated that there will be a greater increase in the proportion of patients with diabetes who are aged 45–64 years (Adiseshiah,2005).The problem of DM in Iraq is progressively increasing and every year large number of population implicate with this dame disease(Abed al-Rahman and Al-Hadith,2013).The prevalence of type 2 diabetes rates continue to increase with increasing number of patients at risk of serious diabetes-related complications. T2DM has a substantial genetic component and is thought to be polygenic in nature (Jenkins *et al*., 2013). Several genes influence the underline level of glucose tolerance and thereby contribute to overall susceptibility to T2DM (Lorenzo *et al*., 2013). Genetic linkage analysis and association studies have identified several candidate genes contributing to T2DM. However, given the ethnic differences in life style, environmental factors as well as in the genetic background, it is important to examine polymorphisms related to T2DM in each ethnic group (Yamada *et al*., 2006; Wilson *et al*., 2007).

It is generally believed that both thyroid disorders and diabetes mellitus are common in many countries(Abdelgadir,2006).The level of

blood glucose is affected by some hormones include: Insulin, glucagon ,growth hormone, cortisol , epinephrine and thyroid hormones(Couri *et al*., 2009).The thyroid gland is palpable in about 50% of women and 25% of men. These hormones regulate protein, fat, and carbohydrate metabolism and affecting how human cells use energetic compounds. There is an abnormal response to glucose tolerance testing in hyperthyroidism because glucose rises faster than normal, and increase insulin degradation(Hoffner,2005).In hypothyroidism liver secretion of glycogen increases absorption of glucose and glucose utilization is slowed(Noe,2002; Simon *et al*., 2008).

Thyroid disorders such as goiter, nodules, and autoimmune thyroid disease and thyroid dysfunction have rarely been investigated in diabetic patients. A number of symptoms and signs are well-established manifestations of thyroid dysfunction. Additional findings in patients, personal and family histories indicate increase risk of developing thyroid dysfunction, diabetes mellitus represent risk factor of developing thyroid dysfunction(Paul *et al*., 2000).Thyroid disorders may be undiagnosed in diabetics because their common signs and symptoms are similar to those of diabetes itself. Uncontrolled diabetes, either T1 or T2, may induce a low T3 so that total T3 and free T3 will decrease and reverse T3 will increase but TSH and T4 will stay normal. In some studies, the prevalence of thyroid disorders in T2 DM has been reported from 12.5% to 19% (Nobre *et al*., 2002; Radaideh *et al*., 2004).

The American Thyroid Association guidelines for type 2 diabetes patients require frequent testing for thyroid dysfunction (Ladenson *et al*., 2000).

Adenosine triphosphate-binding cassette transporterA1 (ABCA1) is a 2261-amino acid integral membrane protein that is highly expressed in the

liver and tissue macrophages. SOURCE Homo sapiens (human) NCBI Reference Sequence: NG_007981.1. rs2066715 *[Homo sapiens]*.

It is a cell membrane transporter and mediates the efflux of cholesterol, phospholipids, and other lipophilic molecules from cells; transfer of these molecules from peripheral cells to lipid-poor Apo lipoprotein A1 is the first step in (HDL) particle formation by a number of metabolically active pathways (Attie *et al*., 2001;Oram and Vaughan, 2006).ABCA1gene has been reported to play an important role in cholesterol metabolism especially in high-density lipoprotein cholesterol HDL-C (Daimon *et al*., 2005;Saleheen *et al*., 2006). Animals and human studies documented that defects in the ABCA1 pathway are significant determinants of coronary artery disease(CAD)(Vergeer *et al*., 2010).The ABCA1 gene is located on the chromosome 9 in the area 9q31.1 and encodes ABCA1 protein. This gene protein is expressed in liver, macrophages, intestines, lungs etc. This gene expression is over expressed by modified LDL(Langmann *et al*., 1999), cAMP(Oram *et al*., 2001;Abe-Dohmae *et al*., 2000), and ox sterols, inhibited as well, which are small molecules that influenceABCA1 levels via signaling pathways involving nuclear receptors such as liver-X receptor (LXR) and persoxisome proliferators activated receptor-gamma (PPAR-g)(Slatter *et al*., 2008;Qiu and Hill, 2008).

1.2 Aim of the study:

The study aimed to determine the correlation between ABC A1 gene polymorphism and lipid in Type 2 diabetes mellitus patients