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CORRELATION BETWEEN ANTHROPOMETRIC PARAMETERS WITH LOW DENSITY LIPOPROTEIN RECEPTOR (A370T) GENE POLYMORPHISM IN TYPE II DIABETES PATIENTS OF IRAQI POPULATION

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ABSTRACT

Background: (A370T) is a SNP in the 370th nucleotide in exon 8 of the low density lipoprotein receptor (LDLR) gene where Guanine (2-amino-6-oxy purine) transported to Adenine (6-amino purine) and this substitution is of transition type as each of Guanine and Adenine are purine nitrogen base. The (A370T) SNP causes an amino acid replacement (Alanine to Threonine) in the translated polypeptide. Aim: This study was designed to assess the relationship between the low density lipoprotein receptor (A370T) single nucleotide polymorphism and different anthropometric parameters in type 2 diabetes mellitus patients in Kerbela city. Materials and Methods: A case-control study of 180 patients (90 male and 90 female) with type II diabetes mellitus. Body mass index, serum lipid profiles and fasting blood sugar were measured in sera of each patient (males and females) and the statistical analysis was made by SPSS 22. Results: The results attained in the current study showed that the fasting blood glucose and glycated hemoglobin were significantly higher ($p<0.001$) in diabetic patients than control subjects. Additionally, data gotten from comparison between males and females within the diabetic patients with dyslipidemia group revealed that females have significantly lower levels of high density lipoprotein cholesterol and higher levels of total cholesterol compared to males ($p<0.05$) . There was no relationship between the low density lipoprotein receptor (A370T) gene polymorphism and the measured parameters in patients with type II diabetes mellitus. The results of this study agreed with several studies which stated that there was no association between the LDLR gene polymorphism (A370T) with dyslipidemia and any of anthropometric parameters and the (A370T) polymorphism doesn't considered as a risk factor for dyslipidemia development in patients with type 2 diabetes mellitus in Iraqi patients. Conclusion: There was no association between the LDLR gene polymorphism (A370T) with dyslipidemia and any of anthropometric parameters at the study groups and the (A370T) polymorphism doesn't considered as a risk factor for dyslipidemia development in patients with type 2 diabetes mellitus in Iraqi patients.

KEYWORDS: Diabetes Mellitus (DM), A30T SNP, LDLR, Dyslipidemia.

INTRODUCTION

Anthropometry refers to the measurement of the human individual for example involves the systematic measurement of the physical properties of the human body, primarily dimensional descriptors of body size and shape. Changes in lifestyles, nutrition, and ethnic composition of populations lead to changes in the distribution of body dimensions (e.g. the rise in obesity) and require regular updating of anthropometric data collections.[1]

Obesity has become a major worldwide health problem. In every single country in the world, the incidence of obesity is raising continuously and therefore, the associated morbidity, mortality and both medical and economical costs are expected to increase as well. The majority of these complications are related to co-morbid conditions that include coronary artery disease, hypertension, type 2 diabetes mellitus, and dyslipidemia. Obesity increases cardiovascular risk through risk factors such as increased fasting plasma triglycerides, high LDL-cholesterol, low HDL-cholesterol, elevated blood glucose and insulin levels and high blood pressure.[2]

Type II diabetes mellitus is associated with a two to fourfold excess risk of coronary heart disease (CHD) and the usual risk factors for coronary artery disease account for only 25–50% of increased atherosclerotic risk in diabetes mellitus. Other obvious risk factor is dyslipidemia which contribute substantially to the increased risk of macro-vascular disease in diabetic patients.[3,4]

Dyslipidemia is an abnormal amount of lipids (e.g. triglycerides, cholesterol and/or fat phospholipids) in the blood. In developed countries, most dyslipidemias are hyperlipidemias; that is, an elevation of lipids in the blood. This is often due to diet and lifestyle. Dyslipidemia is a common feature of diabetes and it is known that diabetic dyslipidemia happens not only as a disruption of lipoprotein metabolism resultant from changes in the quantitative and qualitative features of lipoproteins, but may also progress due to genetic and environmental factors.[5] Dyslipidemia in diabetes commonly manifests as raised low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) levels, or elevated triglyceride (TG) levels.[2] Its seems that a number of factors may contribute to the modifications in lipid metabolism detected in patients with diabetes, including insulin deficiency or resistance adipocytokines, and hyperglycemia.[3] Physicians and basic researchers classify dyslipidemias in two distinct ways:

1. Presentation in the body (including the specific type of lipid that is increased).
2. Underlying cause for the condition (genetic, or secondary to another condition). This classification can be problematic, because most conditions involve the intersection of genetics and lifestyle issues. However, there are a few well-defined genetic conditions that are usually easy to identify.

The LDL receptor gene consists of 18 exons spanning 45 kb on chromosome19p13.The 5.3 kb mRNA encodes a mature protein of 839 amino acids. Overall, more than 800 mutations, including gross deletions, minor deletions, insertions, point mutations, and single nucleotide polymorphism scattered over the LDL receptor gene have been reported.[6] These mutations affect the synthesis (class 1), posttranslational processing (class 2), ligand binding activity (class 3), internalization (class 4), or recycling (class 5) of the LDL receptor.[7]

Mutations in the low density lipoprotein receptor (*LDLR*) result in ineffective clearance of serum low density lipoprotein (LDL) cholesterol and contribute to premature atherosclerosis and cardiovascular disease (CVD) in familial hypercholesterolemia.[8] Several mutations of the *LDLR* have been described, affecting exons, splicing sites and the promoter region, summarized in.[9] Mutations in genes of other proteins involved in LDL uptake and metabolism (ApoB and LDL receptor adaptor protein – LDLRAP1) and in LDLR intracellular recycling (propoprotein convertase subtilisin/kexin type 9 serine protease, PCSK9) have also been implicated in familial hypercholesterolemia.[9] More recently, *PCSK9* mutations associated with reduced serum LDL cholesterol were associated with a lower risk of coronary heart disease (CHD) in the Atherosclerosis Risk in Communities (ARIC) study.[10]

The aim of the presented work is to evaluate the correlation between some of anthropometric parameters such as body mass index, gender with various lipid profiles in type 2 diabetic patients and also to evaluate their correlation with (A370T) Gene Polymorphism.

MATERIALS AND METHODS

The study subjects were 180 candidates (90 Male and 90 Female), with mean age value of (59.19 ± 5.68) years. Sixty subjects serves as control and they were apparently healthy and the other 120 subjects were chosen to have type II DM (60 with dyslipidemia and 60 without dyslipidemia) based on physicians diagnosis prior to the current visit to the outpatient department of Al-Hussein Teaching Hospital, Al-Hussein Medical City / Kerbala Health Directorate / Kerbala - Iraq. Type II diabetes diagnosis was based on fasting blood glucose (FBG) level > 126 mg/dL and/or postprandial glucose level > 200 mg/dL and HbA1c level > 6.5%. Subjects with triglyceride levels > 200 mg/dL and/or HDL-C levels < 45 mg/dL were diagnosed with dyslipidemia.[6]

Serum total cholesterol (TC) was measured by a laboratory kit (BIOLABO/ France 80106) and according to Burits principle.[11] Triglycerides are enzymatically hydrolyzed to glycerol and fatty acids and measured according to Fossati equation by (80019 BIOLABO/ France) kit.[12] The HDL-C fraction was determined by Wilson procedure.[13] LDL-C was determined mathematically from the total cholesterol, triglycerides; and the HDL-C concentration by using Friedwald's formula.[14] VLDL-C was calculated by Ana Vujovic Principle.[15] In the FBS measurement glucose is oxidized by glucose oxidase to gluconate and hydrogen peroxide according to Huggett and Nixon equation using (BIOLABO / France 80009) kit.[16]

Genomic DNA extraction was made by (Bioneer/ Korea K-3032) laboratory kit. The concentration and the purity of the extracted DNA was determined by nano drop device. The process of the (A370T) polymorphism genotyping was done by the amplification of 150 bp region of LDLR gene by Polymerase Chain Reaction (PCR) using two primers:

P1**:** 5'-GAG TGT CAG GAT CCC GAC ACC TGC GCC-3'

P2: 5 '-AAG TCG ACC CAC CCG CCT GCC TCC CGT-3'

The PCR mixture is prepared in lyophilize PCR premix formula by adding:- 1µl of 10 pmol/µl of each primers and 5 µl of extracted DNA and The volume was completed to 20 µl by distilled water. The thermal program comprise of 35 cycles. The PCR products were digested with the enzyme *Hae*III, and fragments were separated on 3.5% agarose gel and visualized by ethidium bromide staining.[17]

The results were expressed as mean ± standard deviation (Mean ± SD). The comparisons between groups were performed with analysis of variance and Student's t-test, using Statistical Package for Social sciences (SPSS)

software 22. Significant: p<0.05, highly significant: p<0.001, No significant: p>0.05.

RESULTS

The obtained data of the molecular analysis was 150 base pair in size and as confirmed by electrophoresis with 25 bp ladder in the digestion process by *HAE III* enzyme. The fragments representing A allele were 77, 47 and 26 bp, and those representing the T allele were 124 and 26 bp.

The results obtained in the current study showed that each of FBS and HbA1c values are significantly higher in diabetic patients than control subjects (p>0.05) as shown in Table.[1]

Table (1): Comparison between Control Group and Diabetic patients Group according to the Measured Parameters using Student T-test.

Parameter	Group	N	Mean	SD	P value
FBS (mg/dl)	Control	60	101.9152	32.37333	P<0.001
	Diabetic patients	120	271.0850	89.42512	
HbA1c (%)	Control	60	5.5950	.64003	P<0.001
	Diabetic patients	120	9.2683	1.88457	
P value derived from student T-test test, Significant: p<0.05, Highly significant: p<0.001, No significant: p>0.05., BMI = body mass index ,FBS = fasting blood sugar, HbA1c = glycated hemoglobin.					

The outcomes attained in the current study showed also that the FBS and HbA1c values are considerably greater in type II DM patients with dyslipidemia as compared with the Type II DM patients without dyslipidemia (Figure1).

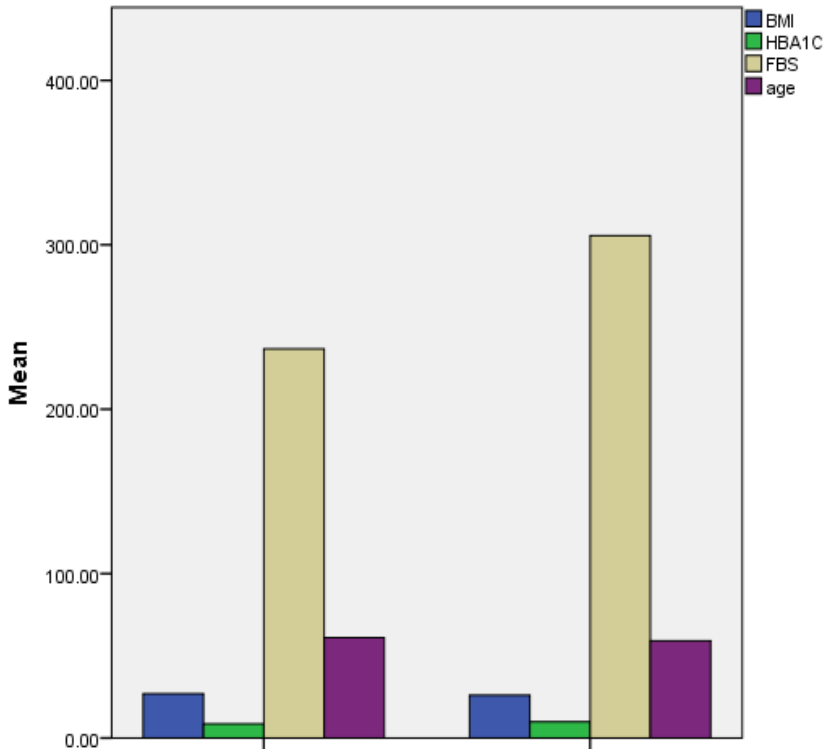

Figure (1): Comparison between type 2 diabetic patients with and without dyslipidemia according to the measured parameters.

The data gained from comparison between males and females diabetic patients with dyslipidemia revealed that females have significantly lower levels of HDL-C and higher levels of total cholesterol compared to males (Figure 2) while there are no significant differences in the other measured biochemical parameters (Table 2).

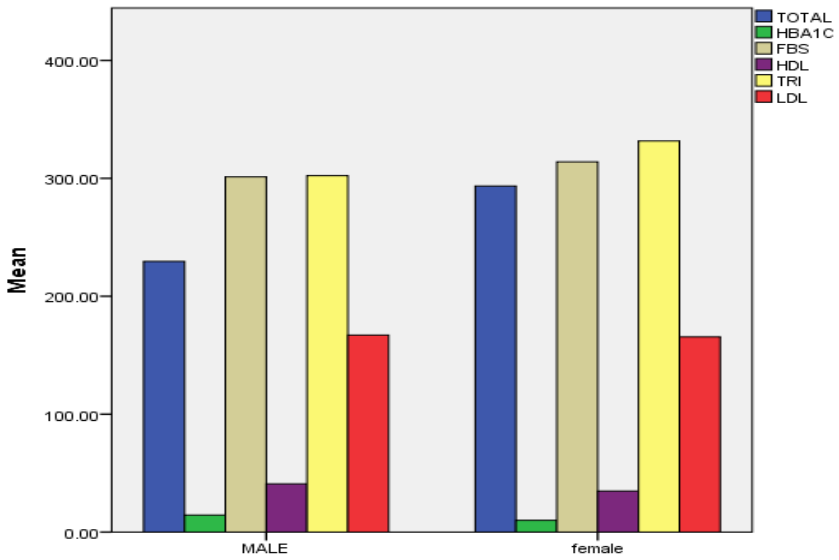

Figure (2): Results of biochemical analysis for the diabetic patients with dyslipidemia group (males' vs females).

Table (2): Results of biochemical analysis for males and females in sera of diabetic patients with dyslipidemia group.

Parameter	Gender	N	Mean	Standard Deviation	P value
FBS (mg/dl)	Male	40	301.3	82.85	N.S
	Female	20	313.96	53.42	
Total cholesterol (mg/dl)	Male	40	229.62	116.29	P=0.05
	Female	20	293.46	117.23	
HDL-C (mg/dl)	Male	40	40.96	9.92	P<0.05
	Female	20	34.82	5.613	
Triglyceride (mg/dl)	Male	40	302.21	112.27	N.S
	Female	20	331.62	90.92	
LDL-C (mg/dl)	Male	40	167.08	62.79	N.S
	Female	20	165.58	58.73	
P value derived from student T-test test, Significant: p<0.05, Highly significant: p<0.001, No significant: p>0.05., FBS = fasting blood sugar, HDL=high density lipoprotein, LDL= low density lipoprotein					

On the other hand, there is no significant association between LDLR gene polymorphism and other measured parameters (p>0.05) as mention in Table (3).

Table (3): Association Between LDLR Genotypes and other Measured parameters.

Parameter	allele	Control	DM patients without dyslipidemia	DM patients with dyslipidemia
BMI (Kg/m²)	AA	23.6217± 3.16838	26.4882 ±3.67040	26.472 ± 3.13492
	AT	23.0833 ± 2.39159	30.1600 ± 3.21605	24.75 ± 3.49489
	TT	23.3500 ±.49497	28.5250 ± 1.51740	24.000 ± 2.000
P. value		N.S	N.S	N.S
FBS (mg/dl)	AA	97.51541±8.92295	232.9353 ± 88.039	304.8191 ± 71.9033
	AT	100.8667±11.8722	276.56 ±119.9730	275.812 ± 76.657
	TT	114.400 ±7.63675	234.175 ± 102.447	359.60 ± 76.4807
P. value		N.S	N.S	N.S
HbA1c (%)	AA	5.5283 ± .61776	8.4588 ± 1.36926	13.5511 ± 19.307
	AT	5.7083 ± .51954	9.7800 ± 2.99783	11.000 ± 2.39046
	TT	6.45001 ± .48492	9.5000 ± 2.54689	11.000 ± 1.58114
P. value		N.S	N.S	N.S
Total cholesterol	AA	153.332 ± 21.8173	161.392 ± 29.3352	262.905 ± 117.934
	AT	156.3250± 23.4828	171.96 ± 29.05724	226.3875 ±148.2532

(mg/dl)	TT	163.80 ± 24.18305	158.00 ± 32.76207	177.260 ± 51.366
P value		N.S	N.S	N.S
HDL-C (mg/dl)	AA	55.7130 ±10.06125	51.8020 ±9.52383	38.8149 ± 9.66790
	AT	54.2250 ±11.07340	50.660 ±11.83841	39.7625 ±7.87907

DISCUSSION

Dyslipidemia is one of the risk factors for development of cardiovascular diseases (CVDs), the leading causes of death in adults worldwide,[4] given its importance, studies are being conducted to determine the abnormalities associated with plasma lipid**.**The existing study outcomes showed that the FBG and Hba1c levels were higher in diabetic patients as compared to control subjects, This conforms the study of Ghazanfari, et al. who presented that FBG and HbA1c are used as diagnostic bio-marker to separate diabetic from non-diabetic subjects.[18] Also the diabetes complications and control trial stated that HbA1c is the gold standard of glycemic control and HbA1c of up to 7.0% may reduce the risk of cardiovascular complications.[19]

The resultant data in the current study showed also that within the local diabetic patients with dyslipidemia, females have significantly higher level of total cholesterol, and significantly lower level of HDL-C as compared to males. This finding links with the previous study showing that diabetic dyslipidemia is more atherogenic than that of normal dyslipidemia and female are more prone to it.[20] This fact may be attributed to the effects of sex hormones on body fat distribution, which leads to differences in changed lipoproteins as the women experience a number of hormonal changes throughout their lifetime, comprising those fluctuations associated with puberty, menarche, pregnancy, and menopause.[21] Each of these hormonal perturbations can alter serum lipoprotein levels and lipoprotein irregularities connected to women's health with menopause; women experience a worsening of their lipid profile, with change to higher and more atherogenic dyslipidemia,[22]

Adverse lipoprotein patterns found in postmenopausal women are thought to be partly responsible for their high CVD risk. The fall of estrogen levels after menopause is certainly responsible for most of these modifications, either through direct effects on lipid metabolism or through the regulation of body composition and energy balance.[23] After menopause, women begin to show a redistribution of body visceral fat and a typical abdominal localization,[24] Estrogen may also directly influence lipid metabolism through the suppression of gene expression and activity of lipoprotein lipase (LPL), the rate limiting enzyme in triglycerides metabolism,[23,25] or through the modulation of lipolysis by the up-regulation of α2- adrenergic receptors. Moreover, it has been shown that estrogen replacement therapy in postmenopausal women decreases the expression of several lipogenesis genes, such as sterol regulatory element binding protein 1c, fatty acid synthase, acetyl-CoA carboxylate, LPL and peroxisome proliferator-activated receptor-γ.[26]

There were many revisions which examine the possible effect of (A370T) genetic form of the LDLR on lipid profile in normal populaces from different countries and origins. The first Iceland studies,[27] stated that males have 370TT (N = 19) has about 8.2% greater total cholesterol level, 11.9% more LDL-C, with 10.4% advanced Apo-B ranks in comparison to AA males (n = 135), while females with 370TT (N = 13) had 7.5%, 13.4% with 10.2% lesser amounts of total cholesterol, LDL-C and Apo-B correspondingly, in comparison to AA females (n = 155). One other large study from Canada,[28] that illustrate neglected variances in each of total cholesterol or LDL-C in approximately 9000 Canadian individuals additional Dual minor studies, the first in Singapore (n = 538),[29] which concluded small occurrence for TT homozygous individual (n = 4), the second study was in Germany (n = 102) which established 12 AT heterozygous topics with just 1 individual with TT form,[30] each of these studies stated no differences of lipid profile in each of the genetic form for the LDLR. other additional study in Denmark stated that subjects have TT genotype may have 81% advanced danger for coronary artery disease with a statistically important 3.7-times advanced danger for ischaemic stroke,[30]

CONCLUSION

From all data and correlations of the different variables in the present study, it could be concluded that:-

1. FBS and HbA1c are all significantly greater in T2DM in comparison to healthy control individuals ($p>0.05$).
2. Females have significantly lower levels of high density lipoprotein cholesterol and higher levels of total cholesterol compared to males ($p<0.05$) within the diabetic patients with dyslipidemia group.
3. There is no relationship between LDLR gene (A370T) polymorphism and the measured parameters in patients with Type II DM.

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