

**ASSOCIATION BETWEEN FAT MASS AND OBESITY ASSOCIATED (FTO) GENE
POLYMORPHISM (RS9939609) AND LIPID PROFILE IN TYPE2 DIABETIC OBESE
IRAQI MALE****Prof. Dr. Fadhil Jawad Al-Tu'ma*¹, Khamail Hassan Obed¹ and Jawad F. Al-Tu'ma²**¹Department of Biochemistry, College of Medicine, University of Kerbala / Holy Kerbala – Iraq.²Al-Zahraa Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorate / Holy Kerbala, Iraq.***Corresponding Author: Prof. Dr. Fadhil Jawad Al-Tu'ma**

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is the most widespread endocrine disease and one of the most acute medical and societal problems, as it leads to early disability and increased mortality from different complications. *FTO* gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase which is involved in DNA repair and fatty acid metabolism. Low-density lipoprotein (LDL- C), high-density lipoprotein (HDL-C), triglycerides, and total cholesterol are heritable, risk factors for CVD, and usually disturbances in plasma lipid levels accompanied T2DM. **Aim:** Investigation the risk of allele frequency of *FTO* gene polymorphism rs9939609 in obese male with type 2 diabetes mellitus and to examine the association of this polymorphism of *FTO* gene with BMI and lipid profile in obese Iraqi male. **Methods:** The study included 120 patients (males) with T2DM and 60 healthy controls to explore the relation of these SNPs with T2DM in obese male in Iraqi population. The patients group was enrolled from Al-Husain medical city in Karbala province based on WHO guidelines of T2DM. BMI, FBS, lipid profile and HbA1c were measured, DNA was isolated from whole blood and the *FTO* gene variant (rs9939609) was genotyped by using ARMS-PCR technique with using specific primers. Multinomial logistic regression was applied to compare the proportions of genotypes or alleles. The odds ratio, T test, and P value at 95% confidence interval (CI) were measured before and after adjustment of BMI and age. Also in the present study the Hardy Weinberg equilibrium was tested. **Results:** The results of our study showed there is no significant differences between the SNP rs9939609 in the *FTO* gene and T2DM in Iraqi obese male, and the genotyping results of rs9939609 was consistent with Hardy–Weinberg equilibrium in obeseT2DM, non obese T2DM, and control individuals ($P = 0.05$), ($P = 0.46$), and ($p = 0.002$) respectively. The results show the studied SNP of *FTO* gene have significant association with BMI and HDL- C level but not have association with other parameters in obese diabetic patients group while It is exhibited failure of *FTO* gene polymorphism to affect any of the measured clinical characteristics in non obese diabetic patients. **Conclusions:** The *FTO* gene polymorphism rs9939609 have significant association with HDL- C level in obese diabetic Iraqi male but does not affected other tested biochemical parameters.

KEYWORDS: Lipid Profile, T2DM, Fat mass and obesity associated (*FTO*) gene, and rs9939609.**INTRODUCTION**

Obesity has become a serious public health issue and in increasingly prevalence in both developed and developing countries (Charo et al, 2014). The main adverse results of obesity are cardiovascular disease, type 2 diabetes, and several cancers (Savini et al, 2013). Type 2 diabetes mellitus (T2DM) is the most common endocrine disease and one of the most acute medical and societal problems, as it lead to early disability and increased mortality from different complications (Wang et al, 2016 ; Lee et al, 2013). T2DM is a multifactorial disease; its pathogenesis is characterized by β -cell dysfunction accompanied by reduction insulin secretion and β -cell mass, a diminished incretin response,

increased glucagon secretion, augmented liver glucose production, enhanced glucose reabsorption, activated lipolysis, reduced glucose absorption by the muscles, and neurotransmitter dysfunctions (Sanghera and Blackett, 2012).

Several studies have shown significant association between adiposity, lipid profiles, dyslipidemia, hypertension, diabetes mellitus or an increased cardiovascular risk and SNPs that have been previously found to influence plasma lipid levels and cardiovascular disease are *APOA1* (rs670), *APOA5* (rs662799), *CETP* (rs1800777), and *FTO* (rs9939609) (Phani et al, 2016; Al-Tu'ma and Obed, 2018). These genes involved in the regulation of plasma lipid levels. Genetic variation and

environmental factors are thought to contribute to the development of T2DM (Sanghera and Blackett, 2012).

The fat mass and obesity-associated *FTO* gene is recognized as associated with enhanced adiposity and appears to influence the risk of obesity in Different nationalities (Tanaka et al, 2013). *FTO* gene is a polymorphic gene located on chromosome 16 at position 16q12.2. This gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase which is involved in DNA repair and fatty acid metabolism. This gene is known as one of the most effective genes in human metabolic pathways with nearly 10,000 variations (Okamoto et al, 2000). The *FTO* protein catalyses the demethylation of single-stranded nucleic acids. The *FTO* mRNA is abundant in many tissues, particularly in the hypothalamic nuclei controlling energy balance. Some researches indicate that the *FTO* gene plays a role in nervous and cardiovascular systems although its biological function is not fully known yet. It is suggested that catalytic activity of *FTO* may regulate the transcription of genes involved in fatty acid and glucose metabolism (Jia et al, 2007).

The catalytic activity of *FTO* may regulate the transcription of genes involved in metabolism by nucleic acid demethylation. It was found that *FTO*-dependent demethylation of specific mRNAs *in vivo* relates to the control of the dopaminergic signaling pathway (Hess et al, 2013). The genetic associations of Human population have shown strong and significant relations between the fat mass and obesity associated gene (*FTO*) polymorphisms and obesity (Daneshpour et al, 2015). However, limited studies are available on the effect of *FTO* variants on lipid concentrations in overweight and obese individuals (Muller et al, 2008 ; Willer et al, 2013), where find that loci associated with blood lipid levels are often associated with cardiovascular and metabolic traits, including coronary artery disease, T2DM, blood pressure, waist-hip ratio, and body mass index (Willer et al, 2013). A meta-analysis of 17,037 white European individuals revealed associations between *FTO* variants not only with BMI, but also with fasting insulin, glucose, triglycerides, and HDL-cholesterol concentrations (Freathy et al, 2008). The influence of ethnic differences is often noted as the cause of these differences (Rees et al, 2011; McCormack and Grant , 2013; Li et al, 2008). Levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides and total cholesterol are heritable, modifiable risk factors for coronary artery disease (Willer et al, 2013).

The aim of this study was to investigate the risk allele frequency of *FTO* gene polymorphism rs9939609 in obese male with type2 diabetes mellitus and to examine the association of *FTO* gene polymorphism with BMI and lipid profile in obese Iraqi male.

MATERIALS AND METHODS

Study subjects: A case- control study which including 180 male, 120 diabetic males and 60 healthy persons as control group, were conducted to assess the association of the (rs9939609) SNP of *FTO* gene with T2DM in obese males in Iraqi society.

Patients group: The patient samples included 120 obese subjects with T2DM divided into 60 obese type2diabetic patients and 60 non obese type2diabetic patients were recruited from Al-Husain medical city / Kerbala - Iraq from January 2017 to December 2017. The cases and control subjects were recruited on the basis following inclusion criteria. For cases: (a) patients those diagnosed by specialized physician as having T2DM according to WHO guidelines, (b) Age of patients ≥ 35 years, (c) FBS > 126 mg/dl, (d) HbA1c > 6.5 , (e) BMI > 30 Kg/m² for obese patients.

The exclusion criteria were: (a) Men those diagnosed with T1DM, (b) Diabetic patients with age < 35 years, (c) Patients suffering from kidney or liver diseases, (d) Patients with Ischemic heart disease, (e) Patients with tumor diseases, (f) Patients whose taken lipid lowering drugs such as statin.

Control group: The control group included 60 healthy persons were selected randomly from the general population who attend the hospital for checkup also from relatives and colleagues.

The exclusion criteria for control group were: (a) Persons with (Ischemic heart disease, acute or chronic kidney or liver diseases, or hypertension), (d) and Persons who intake statin drug.

The inclusion criteria for non-diabetic controls were: (a) males with age at examination ≥ 35 years, (b) fasting blood glucose < 100 mg/dl, (c) HbA1c values $< 6.0\%$, (d) No past medical history of type 2 diabetes, (e) no family history of type 2 diabetes in first and second degree relatives, (f) the rang of BMI is $22 \text{ kg/m}^2 < \text{BMI} < 36 \text{ kg/m}^2$, (g) TC < 200 mg/dl, (h) and TG < 150 mg/dl.

All subjects answer a detailed questionnaire that includes information about family history, the mean age was (35-79) years old, drug history, medical history and other relevant information, for all subjects" weight, height and BMI had measured. All cases were collected from January 2017 to December 2017. It should be noted that Karbala was one of the big cities of Iraq, and there was no much difference in genotyping distribution from one city to another, therefore our study society could represent the Iraqi society. Informed consent has been taken from all subjects. The study protocol was approved by Karbala Medical College Ethical Committee.

Phenotypic data: Height and body mass were used to calculate body mass index. Plasma glucose, serum

triglyceride, HDL-C, LDL-C, VLDL-C, and total cholesterol were determined from the blood.

Genotyping: The blood samples of T2DM and control groups were collected in EDTA-anticoagulated tubes, Genomic DNA was isolated from the whole blood by using the Genomic DNA mini kit (Blood / cultured cell) (Gene aid/ China). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Nano-drop, USA).

Genotyping was carried out using tetra primer amplification refractory mutation system (ARMS- PCR) for FTO gene using the thermocycler (Cleaver, USA). The list of primers sequences and PCR condition used in our study for the SNP (rs9939609) of FTO gene obtained from (Müller et al, 2008) as following:

Fout: 5'-TGG CTC TTG AAT GAA ATA GGATTC AGA A-3'
Rout: 5'-AGC CTC TCT ACC ATC TTA TGT CCA AAC A-3'
Fin: 5'-TAG GTT CCT TGC GAC TGC TGT GAA TAT A-3'
Rin: 5'-GAG TAA CAG AGA CTA TCC AAG TGC ATC TCA-3'

Amplification was performed in a 20µl premix tube, each premix tube containing 0.7 µl from Fout and Rout outer primers which added respectively, 1 µl from each inner primer, 5 µl of extracted DNA, and completed the volume to 20 µl by distilled water. Cycling conditions were 93°C for 5 min followed by 30 cycles of 93°C for 30s, 53c for 25s, 72°C for 25s and the final extension of

72°C for 5min. amplification product of FTO gene was 321 bp. amplification product of FTO gene was run on 1.5% agarose gel.

Statistical analysis: Phenotypes data expressed as mean \pm SD and genotypes data expressed as frequencies, ANOVA test and student T- test used to compare phenotypes data between control group and obese and non obese T2DM groups, and across genotypes using SPSS windows software (SPSS Inc., Chicago, IL). Association analysis of rs9939609 SNP with T2DM was performed using Pearson's Chisquared test. Multi nominal logistic regression analysis was used to further test the association of SNP with T2DM measured by odds ratio (OR) and corresponding 95 % confidence interval (CI) after adjusting for age and BMI as covariates. Association analysis was also performed assuming co-dominant, dominant and recessive models.

RESULTS

The case – control study was conducted on a total of 120 clinically confirmed T2DM subjects (classified into 60 obese T2DM male and 60 non obese T2DM male) and 60 healthy male as control group.

The clinical and biochemical characteristics of the study subjects were presented in (table 1); it revealed significant differences in FBS, Age, HbA1c, BMI, and lipid profile between obeseT2DM group, non obese T2DM group, and control group.

Table 1: Clinical and biochemical characteristics of study subjects.

Parameters	Control N = 60	T2DM Obese N = 60	T2DM Non-Obese N = 60	P value
FBS (mg/dl)	104.71 \pm 17.99	249.85 \pm 85.03	222.9 \pm 81.54	0.001
Age (y)	49.37 \pm 11.86	52.45 \pm 9.92	55.38 \pm 10.95	0.012
BMI (kg/m ²)	28.08 \pm 3.89	31.42 \pm 1.95	23.46 \pm 1.18	0.001
TC (mg/dl)	184.81 \pm 51.41	176.8 \pm 36.87	164.64 \pm 37.91	0.035
TG (mg/dl)	197.06 \pm 94.94	229.95 \pm 106.27	199.55 \pm 93.05	0.127
VLDL-C (mg/dl)	38.48 \pm 19.37	46.7 \pm 20.30	39.4 \pm 18.61	0.04
LDL-C (mg/dl)	120.43 \pm 42.10	104.45 \pm 34.13	100.44 \pm 40.65	0.014
HDL-C (mg/dl)	41.5 \pm 11.95	34.06 \pm 9.43	37.15 \pm 11.31	0.001
HBA1c%	5.17 \pm 0.79	9.39 \pm 1.89	8.95 \pm 2.10	0.001

BMI body mass index, HbA1c glycated hemoglobin, FBS fasting Blood Sugar, TC total cholesterol, TG triglycerides, HDL-C high density Lipoproteins, LDL-C low-density lipoproteins, VLDL-C Very Low Density Lipoproteins- cholesterol Data were expressed as mean \pm SD

Genotyping

Genotypes did not deviate from Hardy–Weinberg equilibrium in obeseT2DM, non obese T2DM, and control individuals (P = 0.05), (P = 0.46), and (p = 0.002) respectively (table 2).

Table 2: Analysis of Hardy–Weinberg equilibrium.

P value	X ²	Subjects
Obese- DM	9.6	0.05
Non-obese- DM	3.56	0.46
Control	16.9	0.002

PCR product of FTO gene polymorphism (rs9939609) was electrophoresed on 1.5% agarose (100 V and 90 min) and then stained with ethidium bromide, later visualized under UV light. Results discovered two bands (321, 210 bp) for TT wild type, three bands (321, 210, 178 bp) for AA homozygous, and two bands (321, 178 bp) for TA heterozygous genotypes, as shown in (Figure 1).

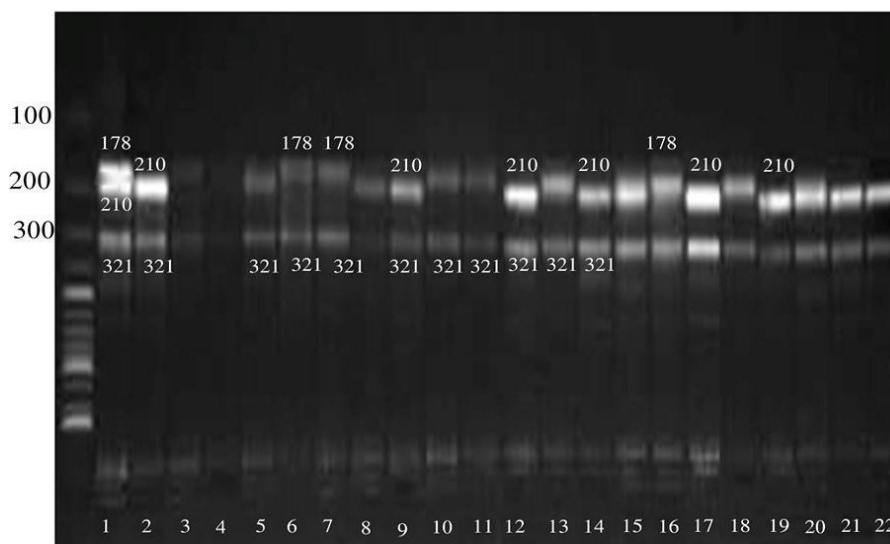


Figure 1: Product of FTO gene polymorphism (rs9939609) analyzed on 1.5% agarose gel electrophoresis.

The Genotype and allele frequencies of *FTO* gene variant are shown there is No statistically significant

association was observed between genotypes of *FTO* gene variant rs9939609 and T2DM (table 3).

Table 3: Genotype and allele frequency of rs9939609 polymorphism of *FTO* gene and association of this variant with T2DM in the study individuals.

Rs9939609 (T/A)	Control N=60	Obese T2DM N=60	Non-Obese T2DM N=60	Unadjusted OR (95% CI) P value	Adjusted OR* (95%CI) P value
Co-dominant					
TT	25	32	28	Reference	
TA	14	16	22	0.893 (0.367-2.16) 0.802	0.723 (0.317-2.36) 0.732
AA	21	12	10	0.446 (0.185-1.07) 0.073	0.521 (0.275-1.23) 0.510
Dominant					
AA+ TA	35	28	32	0.625 (0.30-1.28) 0.202	0.832 (0.42-1.34) 0.921
Recessive					
AA	21	12	10	0.464 (0.203-1.06) 0.069	0.621 (0.324-1.22) 0.060

*For age and BMI

The biochemical characteristics of studied individuals according to *FTO* gene variants rs9939609 show significant association between the studied SNP and BMI, HDL- C in obese diabetic group, while failure of

FTO gene polymorphism to affect any of the measured clinical characteristics in non obese diabetic group. (Table 4, 5).

Table 4: Clinical characteristics of obese T2DM subjects according to *FTO* gene rs9939609 genotype.

Clinical characteristics	TT (N=32)	TA(N=16)	AA (N=12)	P Value
BMI (kg/m ²)	33.607 ± 2.266	31.85 ± 2.245	32.42 ± 2.314	0.031
TC (mg/dl)	172.3 ± 39.61	172.3 ± 35.14	197 ± 27.7	0.119
TG (mg/d)	224.9 ± 113.6	221.6 ± 102.3	258.8 ± 98.64	0.60
VLDL-C (mg/dl)	46.85 ± 20.74	43.95 ± 20.49	51.54 ± 19.72	0.62
LDL-C (mg/dl)	98.5 ± 38	107.9 ± 30.93	113 ± 29.28	0.40
HDL-C (mg/dl)	32.93 ± 7.45	36.42 ± 11.59	41.67 ± 11.73	0.04

Table 5: Clinical characteristics of non obese T2DM subjects according to *FTO* gene rs9939609 genotype.

Clinical Characteristics	AA (N=10)	TA (N=22)	TT (N=28)	P Value
BMI (kg/m ²)	23.535 ± 1.57	23.304 ± 7.64	23.66 ± 0.5	0.72
TC (mg/dl)	164.535 ± 44.854	162.91 ± 31.77	169.44 ± 31.68	0.86
TG (mg/dl)	184.428 ± 90.930	204.26 ± 85.126	234.55 ± 117.40	0.22
VLDL-C (mg/dl)	36.857 ± 17.456	39.652 ± 17.468	46.67 ± 23.34	0.22
LDL-C (mg/dl)	97.248 ± 46.431	98.17 ± 32.19	116.22 ± 41.63	0.26
HDL-C (mg/dl)	37.035 ± 10.112	39.13 ± 13.44	32.44 ± 8.094	0.14

DISCUSSION

In this study observed that there are lipid abnormalities and significant correlations when compared between Clinical and biochemical characteristics of the three groups obese T2DM, non obese T2DM, and control group with p values ($P < 0.05$) in FBS, BMI, Age, HbA1c, total cholesterol, High Density Lipoprotein (HDL-C), Low Density Lipoprotein (LDL-C), and Very Low Density Lipoprotein (VLDL- C) but there is no significant association with TG ($P > 0.05$), this results inconsistent with the findings of (Laith *et al.*, 2017). And this abnormalities including, elevated triglyceride, decrease high density lipoprotein (HDL-C) and increase Very Low Density Lipoprotein (VLDL-C) in obese T2DM patients consistent with the findings of the study of (Hanish. R *et al.*, 2016). Such results can be explained through the fact that individuals with type2 diabetes have an increased prevalence of lipid profile abnormalities which contributes to higher rates of CAD (American Diabetes Association, 2014). Such results coordinate with the findings of (Al Tae, 2009).

For our knowledge this is the first study to investigate the allele frequency and genotype distribution of *FTO* gene rs9939609 and its association with disturbances of serum lipids in Iraqi male population.

Biochemical characteristics (BMI, cholesterol, TG, VLDL-C, LDL-C, and HDL-C) of T2DM obese patients were analyzed with respect to the distribution of the genotypes. Genotypes of the *FTO* gene were considered only due to the No significant changes of this SNP with respect to type 2 diabetes mellitus that were obtained.

Results of our study show significant association BMI ($p = 0.031$), and HDL value ($p = 0.04$).but no significant association with other biochemical parameters (TC, TG, LDL- C, and VLDL- C) in obese diabetic group with TT, TA, and AA genotypes. This came in contrast with other studies which showed non-significant association with all lipid profile (Xi B *et al.*, 2010; Sentinelli *et al.*, 2012) or showed association with elevated triglycerides levels (Zabena *et al.*, 2009; Freathy *et al.*, 2008). However, it came in agreement with other studies that reveal decrease HDL-C levels (Zabena *et al.*, 2009). Moreover, the associations in other studies were more common in men (Lappalainen *et al.*, 2010). Subjects with AA genotype have higher BMI compared to TA and TT carriers, however this difference didn't reach statistical significance, may be due to relatively small sample size

and different effect size in case of BMI. This is in agreement with three previous studies that didn't detect association of *FTO* polymorphism (rs9939609) with BMI in a similar sample size (Zabena *et al.*, 2009). On the other hand, other studies showed that *FTO* risk allele was associated with a significant increase in BMI (Ewens *et al.* 2011).

In the present study there were no significant differences between the three genotypes of *FTO* rs9939609 polymorphism in the non obese diabetic group in tested biochemical parameters (total cholesterol, BMI, LDL-C, HDL-C, and plasma glucose level, and triglycerides). this results agreed with findings of other study that appeared there is no significant association between *FTO* gene polymorphism with BMI, blood glucose concentration, and lipid profile (Freathy *et al.*, 2008), also other investigators, including (Wehr *et al.* 2010; Kowalska *et al.*, 2012) found no significant association between *FTO* rs9939609 different genotypes and any of the lipid profile parameters. Anti-atherogenic action of HDL results from its anti-inflammatory and antioxidant properties. Among the causes of lower HDL-C concentration were: overweight and obesity, physical inactivity, smoking, type 2 diabetes, elevated triglyceride concentration and genetic factors. The latter one is responsible for about 50% of the variability of HDL-C concentrations. Data from the United Kingdom Prospective Diabetes Study suggest that both reduced HDL-C and increased LDL-C predict CVD in diabetes mellitus (Sarat Chandra *et al.*, 2014). According to those available studies the presence of risk allele leads to a decreased HDL concentration. In 2008 Freathy *et al.* reported such association in a large group of Europeans (Freathy *et al.*, 2008). This association was also showed in studies on patients with abnormal glucose metabolism (Lappalainen *et al.*, 2011). A large meta-analysis confirmed the significant relation of the rs9939609 polymorphism with T2DM risk, where the frequency of distribution of rs9939609 among different ethnicities and its proposed association with BMI, insulin resistance and lipid Profile (Ramya *et al.* 2013).The limitation factors of this study could be relatively limited number of subjects in our groups and lack of matched control group due to cost and time limitation.

CONCLUSION

In conclusion, our findings suggest that *FTO* variant rs9939609 is not associated with the susceptibility of T2DM but associated with obesity throw its effect on

BMI. Our study also provides the evidence in support of the adiposity effect of this variant in HDL- C level in Iraqi obese male, and these results accordance with previous findings concerning an influence of *FTO* gene variants on HDL- Cholesterol concentration. Further investigation on larger sample size is required.

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