

**EFFECT OF OBESITY ON INSULIN RESISTANCE AND HBA1C IN TYPE 2 DIABETIC PATIENTS AND ITS RELATIONS WITH FTO GENE SNP (RS9939609)****Dr. Fadhil Jawad Al-Tu'ma\*<sup>1</sup>, Dhuha Haddawi Al-Safah<sup>2</sup> and Jawad Fadhil Al-Tu'ma<sup>3</sup>**<sup>1</sup>Department of Biochemistry, College of Medicine, University of Kerbala / Kerbala - Iraq.<sup>2</sup>Department of Chemistry, College of Science, University of Kerbala / Kerbala - Iraq.<sup>3</sup>Department of Internal Medicine, Al-Zahraa Teaching Hospital, Imam Al-Hussein Medical City, Kerbala Health Directorate / Kerbala - Iraq.**\*Corresponding Author: Dr. Fadhil Jawad Al-Tu'ma**

Department of Biochemistry, College of Medicine, University of Kerbala / Kerbala - Iraq.

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**ABSTRACT**

**Background:** Obesity is increasingly common among Iraqi individuals and shows a significant burden on public health, social and economic development. Environmental and genetic factors are both prominent mechanisms in the pathogenesis of obesity. The understanding of the pathogenesis of the disease is essential to improve the plan of management. Fat Mass and obesity Associated Gene is one of the factors to development obesity where it's located in chromosome 16. SNP were selected in the current study to see its association with obese type 2 diabetes mellitus (T2DM). **Aim:** To detect the effects of obesity of type 2 diabetes mellitus on each of insulin resistance and HbA1c levels and its association with Fat Mass and Obesity associated (*FTO*) rs9939609 variant T2DM of Iraqi population. **Materials and Methods:** This cross-sectional study in which *FTO* gene variant rs9939609 was genotyping in a total 180 male subjects, 92 subjects of them were obese with T2DM and the other 88 subjects were obese without T2DM during Nov., 2017 to Sep., 2018 and both age were matched between the range 40-70 years. The patient's group was enrolled from Al-Husain medical city in Kerbala province based on WHO guidelines of T2DM. Body mass index (BMI), fasting blood sugar (FBS), lipid profile, HbA1c, insulin level and HOMA-IR were measured; DNA was extracted from whole blood and 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. Also in the present study, the Hardy Weinberg equilibrium was tested. **Results:** The results show that each of BMI and HDL-C have a significant association with *FTO* gene SNP (rs9939609), but no other associations with other parameters in obese diabetic patients group,  $p=0.001$ . The results also showed there are no significant differences between the SNP rs9939609 in the *FTO* gene and T2DM and there was no any significant association between *FTO* rs9939609 common variant and insulin level, HOMA-IR. The mean of fasting blood sugar, fasting insulin level, HOMA-IR, TC, TG, LDL-C, VLDL-C and HbA1c are higher in individuals with genotype (AA) and (AT) compared to those with genotype (TT), and the genotyping results of rs9939609 was consistent with Hardy-Weinberg equilibrium in obese T2DM ( $P=0.102$ ), but in obese without T2DM individuals was deviated ( $P=0.003$ ). Minor allele frequency (A) was 0.53 higher in T2DM when compared with non T2DM group. **Conclusion:** The BMI and HDL-C have a significant association *FTO* gene polymorphism rs9939609 in obese diabetic Iraqi male and non-association between insulin level and HOMA-IR with variant of *FTO* gene SNP studies.

**KEYWORD:** T2DM, fat mass and obesity-associated (*FTO*) gene, rs9939609, HOMA-IR.**INTRODUCTION**

Obesity is a risk agent for type 2 diabetes mellitus (T2DM) and enhance insulin resistance and blood glucose levels possible lead to uncontrolled of T2DM.<sup>[1]</sup> Two metabolic defects that lead to T2DM: impairment of each of insulin action and of  $\beta$ -cell function and the action of insulin in insulin-sensitive tissues such as liver, muscle and adipose tissue (insulin resistance).<sup>[2]</sup> The development of type 2 diabetes is caused by a combination of lifestyle, genetic factors and medical

conditions.<sup>[3]</sup> Obesity (BMI  $\geq 30$ ) account for 80-85% of the risk of developing T2DM, where recent research suggests that obese people are up to 80 times more likely to develop T2DM than those with a BMI  $\leq 22$ .<sup>[4]</sup> On the other hand, genetic change assists to the development of T2DM. The greatest advance in the identification of genetic agent fundamental T2DM has been obtain using genome-wide association studies (GWAS) in different populations. More than 100 genetic variants are currently thought to be associated with the risk of development

T2DM.<sup>[5]</sup> Genome-wide association studies have recognized several common genetic variants associated with obesity and diabetes mellitus, one of these the fat mass and obesity-associated gene (*FTO*) variants were found to be consistently associated with obesity-related traits in several populations.<sup>[6]</sup> *FTO* is an enzyme that affects development of human obesity and energy homeostasis, Also known as alpha-ketoglutarate-dependent dioxygenase.<sup>[7]</sup> *FTO* is a polymorphic gene which is located on chromosome 16 and its molecular weight is 58 kDa.<sup>[8]</sup> The *FTO* gene is highly polymorphic, and several polymorphisms of the gene have been found to be associated with obesity or obesity phenotypes, such as high body mass index (BMI). One of these genetic variants (rs9939609), located within the first *FTO* intron, has been related to an increased risk for both obesity and T2DM.<sup>[9]</sup> The *FTO* gene is highly verbalized in the hypothalamus region, which is involved in appetite regulation. The A risk allele in the rs9939609 polymorphism has been associated with increased fat and carbohydrate consumption. This polymorphism has also been associated with a high energy intake, both in adults and children but with no control on energy expenditure. The presence of the a risk allele also look to reduce postprandial satiety.<sup>[10]</sup> On the other hand, study showed that *FTO* gene has a rapid turnover in the pancreatic  $\beta$  cells, involved in the regulation of insulin secretion under glucose stimulation where indicated that *FTO* plays an important role in the biological function regulation of pancreatic  $\beta$  cells. However, the functional role of *FTO* in pancreatic  $\beta$  cells as well as the related molecular mechanism is still unclear.<sup>[11]</sup> The aim of this study was to detect any association of Fat Mass and Obesity associated (*FTO*) rs9939609 variant (mutation of allele T to allele A) with metabolic and anthropometric parameters and insulin level in Kerbala obese men with T2DM.

## MATERIAL AND METHODS

This across-sectional study in which *FTO* gene variant rs9939609 was genotyping in a total 180 male subjects,

**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 ATCTCA-3'

Amplification was performed by addition 10  $\mu$ l master mix, 1.5  $\mu$ l MgCl<sub>2</sub>, 1.5  $\mu$ l from each primer, 5  $\mu$ l of extracted DNA in PCR tube, and completed the volume to 25  $\mu$ l by distilled water. Cycling conditions were 93°C for 5 min followed by 30 cycles of 93°C for 30 s, 53 cycles of 72°C for 25 s, and the final extension of 72°C for 5 min. Amplification product of *FTO* gene was 321 bp. Amplification product of *FTO* gene was run on 1.5% agarose gel by using ethidium bromide stain.

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 diabetic

92 subjects of them were obese with T2DM and the other 88 subjects were obese without T2DM during Nov., 2017 to Sep., 2018 and both age were matched between the range 40-70 years. The patient's group was enrolled from Al-Husain medical city in Kerbala province based on WHO guidelines of T2DM. All participants gave written informed consent after approval of the ethical committee. The inclusion criteria for selecting obese participations were: BMI  $\geq$ 30 Kg/m<sup>2</sup>, FBS > 126 mg/ dl in T2DM and FBS <126 mg/ dl in non-diabetic obese, no family relationship between the subjects with non-diabetic group, no specific disease or chronic history. The exclusion criteria included the participants had no history of kidney disease or using cortisol and lipid lowering drugs.

Five milliliters of venous blood were collected from all individuals participated in this study. The blood was divided into two parts: The first part was used for molecular analysis. It included two milliliter of blood collected in EDTA containing tube and used for DNA extraction, then were analyzed directly to obtain high purity of DNA. The second part included three milliliters of blood placed in serum tube. Blood was centrifuged for 15 minutes at 3000 x g. Serum was collected then stored at -20°C serum that used for determination of different biochemical parameters such as Glucose, TG, HDL-C, LDL-C, VLDL-C, TC and serum insulin.

DNA was isolated from the whole blood by using the Genomic DNA mini kit (Geneaid /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-polymerase chain reaction (ARMS-PCR) for *FTO* gene using the thermocycler (Cleaver, USA). The list of primers sequences and PCR condition used in current study for the SNP (rs9939609) of *FTO* gene obtained from Muller<sup>[12]</sup>, as following:

and non-diabetic groups using SPSS windows software version 23 (SPSS Inc., Chicago, IL). Genotype frequencies were tested for Hardy-Weinberg equilibrium by  $\chi^2$  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) as covariates. Association analysis was also performed assuming co-dominant, dominant and recessive models.

**RESULTS**

The clinical and biochemical characteristics of study individuals are presented in table 1. It revealed

significant differences in FBS, HbA1c, HOMA-IR, and serum insulin between two groups.

**Table 1: Clinical and biochemical characteristics of study subjects.**

Parameters	Obese T2DM, N=92	Obese without T2DM, N=88	P Values
Age (year)	53.26 ± 8.63	53.18 ± 9.25	0.959
BMI (kg/m <sup>2</sup> )	34.78 ± 2.50	34.26 ± 2.56	0.196
FBS(mg/dl)	223.62 ± 43.08	97.93 ± 6.48	< 0.001
Total Cholesterol (mg/dl)	241.00 ± 20.34	240.08 ± 12.76	0.738
Triglycerides (mg/dl)	243.46 ± 42.11	231.27 ± 38.78	0.057
HDL-cholesterol (mg/dl)	37.90 ± 5.21	39.17 ± 4.74	0.92
LDL-cholesterol (mg/dl)	153.56 ± 25.89	152.31 ± 14.39	0.71
VLDL-cholesterol (mg/dl)	48.69 ± 8.22	46.25 ± 7.75	0.57
Insulin(μU/ml)	10.45 ± 4.00	14.12 ± 4.95	< 0.001
HOMA-IR	5.77 ± 2.53	3.42 ± 1.23	< 0.001
HbA1c	9.88 ± 1.62	5.31 ± 0.73	< 0.001

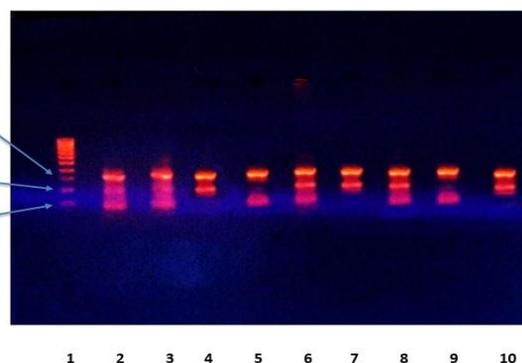
BMI, body mass index; HbA1c, glycated hemoglobin; FBS, fasting blood sugar; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; VLDL-C, very low density lipoproteins cholesterol. Data were expressed as mean ± SD. P < 0.05 is considered as significant level.

Genotypes did not deviate from Hardy–Weinberg equilibrium in obese T2DM individuals (p = 0.102), but in obese non T2DM individual (P = 0.003) as shown in table 2.

**Table 2: Analysis of Hardy–Weinberg equilibrium.**

Subjects	$\chi^2$	P value
Obese diabetic	2.671	0.102
Obese non diabetic	8.732	0.003

**PCR Product:** The amplification product of FTO gene polymorphism (rs9939609), the amplicon size is 321 bp it is described by Müller.<sup>[12]</sup> Results of this study is shown in figure 1.



**Figure 1: PCR- ARMS analysis of the FTO gene by the rs9939609 in obese individuals (with T2DM and without T2DM). The wild type homozygote (TT) showed 2 bands (321, 210) bp, heterozygote (TA) showed 3 bands (321,210,178) bp, homozygote (AA) showed 2 bands (321, 178) bp. The product was electrophoresed on 1.5 % agarose gel at 70 volts for 90 min, stained with ethidium bromide, and then visualized under U.V light (Ladder = 100-1000) bp.**

The Genotype and allele frequencies of *FTO* gene variant are shown in 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.**

SNP rs 9939609 (A/T)	Non-T2DM N = 88	T2DM N = 92	Not adjusted OR (95%CI)	P-Value
<b>Codominant</b>				
TT(Reference)	32	24		
TA	30	38	1.69 (0.83-3.45)	0.14
AA	26	30	1.45 (0.73-3.24)	0.25
<b>Dominant</b>				
AA+TA	56	68	1.62 (0.86-3.06)	0.136
<b>Recessive</b>				
TT+TA(Reference)	62	62		
AA	26	30	1.15 (0.61-2.17)	0.657
Minor Allele frequency	46.65%	53.26%		

OR: Odd Ratio; p<0.05 statistically significant

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

Clinical characteristics	TT N=24	TA N=38	AA N=30	P value
Age (year)	52.70 ± 8.90	52.50 ± 8.29	54.75 ± 8.97	0.54
BMI (kg/m <sup>2</sup> )	32.41 ± 1.05	34.68 ± 2.20	35.60 ± 2.48	0.001
FBS (mg/dl)	214.17 ± 39.66	231.49 ± 45.61	221.04 ± 41.89	0.28
TC (mg/dl)	235.23 ± 21.11	241.30 ± 18.47	245.54 ± 21.56	0.19
TG(mg/dl)	235.46 ± 41.12	245.32 ± 43.64	247.79 ± 37.93	0.52
HDL- C (mg/dl)	40.64 ± 6.22	38.58 ± 5.78	37.34 ± 4.65	0.10
LDL- C(mg/dl)	147.50 ± 28.95	153.65 ± 23.05	158.64 ± 26.60	0.30
VLDL- C(mg/dl)	47.09 ± 8.22	49.06 ± 8.72	49.55 ± 7.58	0.52
Insulin(μU/ml)	10.05 ± 3.16	10.21 ± 4.45	11.10 ± 4.05	0.58
HOMA-IR	5.23 ± 1.59	5.83 ± 2.75	6.15 ± 2.85	0.42
HbA1c	9.63 ± 1.63	9.89 ± 1.66	9.75 ± 1.48	0.82

p<0.05 statistically significant

**Table 5: Clinical characteristics of obese T2DM subjects according to FTO gene rs9939609 genotype by t-test statistic.**

Clinical characteristics	TT 24	AA+TA 68	P value
Age	52.70 ± 8.90	53.45 ± 8.59	0.71
BMI(kg/m <sup>2</sup> )	32.41 ± 1.05	35.65 ± 2.31	0.001
FBS(mg/dl)	214.17 ± 39.66	227.06 ± 44.05	0.21
TC (mg/dl)	235.23 ± 21.11	243.10 ± 19.79	0.10
TG (mg/dl)	235.46 ± 41.12	246.37 ± 41.02	0.26
HDL- C (mg/dl)	40.64 ± 6.22	38.05 ± 5.33	0.05
LDL- C (mg/dl)	147.50 ± 28.95	155.77 ± 24.55	0.18
VLDL- C (mg/dl)	47.09 ± 8.22	49.27 ± 8.20	0.26
Insulin(μU/ml)	10.05 ± 3.16	10.59 ± 4.28	0.57
HOMA-IR	5.23 ± 1.59	5.97 ± 2.78	0.22
HbA1c	9.63 ± 1.63	9.83 ± 1.58	0.59

**Table 6: Clinical characteristics of obese without T2DM subjects according to FTO gene rs9939609 genotype.**

Clinical characteristics	TT N=32	TA N=30	AA N=26	P value
Age (year)	52.63 ± 9.41	54.78 ± 9.29	52.05 ± 9.19	0.59
BMI(kg/m <sup>2</sup> )	32.80 ± 1.80	34.95 ± 2.65	35.55 ± 2.17	0.001
FBS(mg/dl)	95.82 ± 5.50	96.44 ± 5.67	97.14 ± 2.93	0.10
TC (mg/dl)	237.78 ± 13.55	241.55 ± 12.84	242.02 ± 11.30	0.43
TG (mg/dl)	216.90 ± 13.65	228.00 ± 40.90	233.06 ± 36.54	0.11
HDL- C(mg/dl)	41.40 ± 4.59	42.46 ± 6.06	40.45 ± 6.24	0.51
LDL- C(mg/dl)	155.59 ± 11.95	149.89 ± 15.03	149.95 ± 16.86	0.26
VLDL- C(mg/dl)	40.78 ± 2.73	42.20 ± 8.18	42.61 ± 7.30	0.61
Insulin(μU/ml)	12.63 ± 4.30	13.92 ± 4.98	14.30 ± 5.40	0.58
HOMA-IR	3.12 ± 1.01	3.56 ± 1.17	3.67 ± 1.40	0.47
HbA1c	5.24 ± 5.24	5.30 ± 5.30	5.44 ± 5.44	0.67

p<0.05 statistically significant

## DISCUSSION

The two selected groups were found to be different with respect to FBS and HbA1c. The mean values of both the variables (FBS, HbA1c and HOMA-IR) are significantly higher in the diabetic compared to that of without diabetic (Table 1). The two groups do not seem to be differing with respect to the rest of the variables, body Mass Index, lipid profile. Both the groups exhibit means BMI, TC, TG, LDL-C VLDL-C value higher than the normal range, whereas except for HDL-C, which is within the lower than the normal range. Interestingly the presently studied populations exhibit similar distribution, with BMI and age. FBS is found to be significantly higher among the diabetic group compared to non-diabetic. So, we observed that there is significant correlation when compared between clinical and biochemical characteristics of the two groups obese T2DM, obese non- T2DM group with P-values ( $P < 0.05$ ) in insulin, HOMA-IR, HbA1c, but there is no significant association with other parameters (cholesterol, TG, HDL-C, LDL-C, VLDL-C) ( $P > 0.05$ ). This result is consistent with the findings.<sup>[13,1,14]</sup> The results show significant differences among the codominant genotypes models and BMI ( $p=0.001$ ) only. but no significant association with other biochemical parameters while in dominant genotype models show a significant difference with BMI ( $P=0.001$ ) and HDL ( $p=0.05$ ). Our results demonstrate in the Karbala population a strong association between rs9939609 SNP of the FTO gene and BMI in concordance with previously published studies in other Italian populations,<sup>[15]</sup> China,<sup>[16]</sup> India.<sup>[17]</sup> A significant difference in BMI showed in genotype TA and AA groups of rs9939609 higher than the TT group ( $P < 0.05$ ), indicating that the rs9939609 SNP was correlated with the obesity in Iraqi males and this result agreed with Iraqi studies have shown that there is a relationship between rs9939609 and BMI.<sup>[13,18,19]</sup> In this study, we were unable to show any significant association between FTO rs9939609 common variant and insulin resistance. It should be noted that although not statistically significant, fasting glucose level, fasting insulin level, HOMA-IR, TC, TG, LDL-C, VLDL-C and HbA1c are higher in individuals with genotype (AA) and (TA) compared to those with genotype (TT). Our results were identical with another study conducted in Karbala by.<sup>[19]</sup> Others also showed that there was no association between FTO rs9939609 polymorphism and biochemistry parameters such as HOMA-IR, serum insulin levels and blood sugar in obese female adolescents in Indonesia.<sup>[20]</sup> But other work found statistically significant difference polymorphism of rs9939609 in FTO gene with insulin level, HOMA, FBS, HbA1c, and TG in Iranian Women.<sup>[1]</sup> The discrepancy in findings concerning the association between FTO rs9939609 variants and insulin resistance among other studies including our results indicates that the effect of FTO rs9939609 variants on insulin resistance may be influenced by other variables including: gender, age and ethnic.<sup>[21]</sup> The differences in our results may also be due to the limitations created by the small sample population.

## CONCLUSION

In conclusion, our findings suggest that FTO variant rs9939609 is not associated with T2DM, but associated with obesity through its effect on BMI. Our study also provides the evidence in support of the adiposity effect of this variant in HDL-C level in Karbala obese male, and these results accordance with previous findings concerning an influence of FTO gene variants on HDL-C concentration. Further investigation on larger sample size is required. We also did not notice any correlation between this SNP with insulin resistance represented by HOMA-IR.

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