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Original article

Frequency distribution and association of Fat-mass and obesity (FTO) gene SNP rs-9939609 variant with Diabetes Mellitus Type-II population of Hyderabad, Sindh, Pakistan

Farheen Shaikh [a], Tazeen shah [b], Norah Abdullah Bazekh Madkhali [c], Ahmed Gaber [d,e], Walaa F. Alsanie [e,f], Sanum Ali [g], Shafaq Ansari [h], Muhammad Rafiq [i,*], R.Z. Sayyed [j], Nadir Ali Rind [k], Khalid Hussain Rind [k], Akhtar Hussain Shar [k], Syed Mohammed Basheeruddin Asdaq [l]

[a] Department of Biochemistry, Peoples University of Medical and Health Sciences for Women, Shaheed Benazir Abad, Sindh, Pakistan
[b] Department of Physiology, Liaquat University of Medical and Health Sciences, Jamshoro, (LUMHS) Sindh, Pakistan
[c] College of Nursing, Jazan University, Jazan, Saudi Arabia
[d] Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[e] Center of Biomedical Sciences Research (CBSR), Taif University, P.O. Box, 11099, Taif 21944, Saudi Arabia
[f] Department of Clinical Laboratories Sciences, The Faculty of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[g] Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre, Karachi, Pakistan
[h] Department of Physiology, Muhammad Medical College, Mirpurkhas, Sindh, Pakistan
[i] Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro 76080, Sindh, Pakistan
[j] Asian PGPR Society for Sustainable Agriculture, Auburn University, Auburn, AL 36830, USA
[k] Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad, Sindh, Pakistan
[l] Department of Pharmacy Practice, College of Pharmacy, AlMaarefa University, Dariyah, 13713, Riyadh, Saudi Arabia

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ABSTRACT

Background/Aim: Diabetes Mellitus (DM) is one of the important public health issues worldwide. The Fat mass obesity (FTO) gene rs-9939609 variant identified single nucleotide polymorphism (SNP) with the T to A missense mutation, and has a strong association with T2DM. FTO gene is present on chromosome "16q12.2" comprising of nine exons. FTO gene rs-9939609 a variant is commonly found in the Pakistani Population. The purpose of the study was to alert the population about the rs-9939609 variant SNP, having a strong association with T2DM.

Material and Methods: Total of 190 participants were included in the present cross-sectional study. To collect the samples non-probability convenience technique was used. subjects were recruited and divided into three groups, normal healthy subjects, obese and T2DM. The patients were selected from the Medicine department Jamshoro/Hyderabad by filling the pre-designed proforma, as well as verbal and written consent taken from study participants. To analysed the data ANOVA Post hoc (Tukey-test) was applied for comparison among groups ($P < 0.05$) and "SNP-STAT" online software was used for frequencies.

Results: The BMI, neck circumference, waist circumference and lipid profile, fasting blood sugar and HbA1c was found significant ($p < 0.001$) in both genders as compared to control. Homozygous and heterozygous distribution of allelic and genotyping frequency was found in study participants. 37.9 % T/A, 57.4% T/T, and A/A were 4.7%. The FTO gene rs-9939609 variant amplified and have an increased risk

* Corresponding author at: Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro 76080, Sindh, Pakistan.
E-mail address: m.rafiq@usindh.edu.pk (M. Rafiq).

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of developing T2DM in the Sindh population. Codominant model odd ratio of T/A showed 2.42 (*CI*)1.23–3.84, with significant p < 0.032.

Conclusion: The present study concluded that the *FTO* gene SNP rs-9939609 variant was found in the population of Hyderabad, Sindh and having strong association with T2DM and obese individuals. Increase BMI, neck and waist circumference are the biomarkers of obesity and causative factors of T2DM.

1. Introduction

Diabetes Mellitus (DM) is one of the most common public health issues worldwide (GÜNGÖR et al., n.d.; Al-Waili, 2013). More than 300-million people suffered from DM all over the world. Over few decades Pakistan faces socio-economic challenges and much devastating health (Karamanou, 2016; Meo et al., 2016; Meo, 2017). Pakistan possess the 2nd ranked in Diabetes mellitus (DM) out of 21 countries (Adnan & Aasim, 2020), and 9th rank in obesity in the world. Diabetes Mellitus Type- 2 (T2DM) is more common than Diabetes mellitus Type -1 (T1DM). T2DM is characterized by persistent hyperglycemia due to dysfunction of pancreatic β-cell of Langerhans (Reaven & Reaven, 2018). The *FTO gene* is also called alpha-ketoglutarate dependent dioxygenase, which is unified with increased Basal metabolic rate (BMR) and also showing strong relation with T2DM (Yajnik et al., 2009). There are 9 exons of the *FTO* gene with many single nucleotide polymorphisms (SNPs), on chromosome "16q12.2". The SNP (missense mutation) was found at rs9939609 with (53786615) nucleotide position, which shows association with T2DM (Moghanloo et al., 2018). There are many SNP of FTO genes are amplified and contribute to obesity and T2DM but SNP rs-9939609 having the strongest genetic susceptibility with T2DM in the Asian population (Ursu et al., 2015). The researcher tries to the recognition of specific gene variants for T2DM for early detection of T2DM. Boyle et al., (2017), reported that the genetic architecture of Mendelian theory about the polygenic form of obesity was significantly extended. The causative factors of obesity and T2DM are influenced by environmental effects and a sedentary lifestyle. Some researcher also focused on oxidative stress and inflammatory factors which disrupt the pancreatic normal beta-cell function results in insulin resistance (Hurrle & Hsu, 2017). The blood sugar level increased persistently causes the production of free radicles (ROS), which results in oxidative stress which may damage or confirmation change occurred in the receptors due to inhibiting the transcriptional factors (Marinho et al. 2014). The DNA activity for a particular receptor was impaired due to free radicles. The dysfunction of cellular detoxification and scavenging activity lead to genome mutations. The aim of the present study was to analysed and determine the frequency of the FTO gene SNP rs-9969603 variant and its association with T2DM in the population of Hyderabad.

2. Material and Methods

2.1. Study design and participants

A total of 190 subjects were recruited in the present cross-sectional comparative study. This study was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, Pakistan. The sample size was calculated by using Rao software with 11.77% T2DM (Shaikh et al. 2019) by using the proportion of 95% confidence interval and 5% margin of error, the sample size was stand to be n = 159 subjects. Non– probability convenience technique was used. 190 subjects were recruited and divided into three groups. Group A: n = 50 Normal healthy individuals as control then groups further subdivided into two on gender basis A1 (n = 25) females and A2 (n = 25) males, Group B: n = 75 Obese subjects as cases further subdivided into two B1 (n = 35), females and B2 (n = 35) males, whereas Group C: n = 75 T2DM subjects as cases further subdivided into two C1 (n = 35), females and C2 (n = 35) males. Participants were selected on the basis of BMI (Asian Population guidelines). The weight between 18–22.9 kg/m^2 considered as normal, 25–29.9 kg/m^2 as overweight and more than 30 kg/m^2 categorized into obese. The T2 Diabetic patients were recruited from the Medicine department LUMHS, Jamshoro/Hyderabad, Sindh, whereas obese and healthy participants were recruited from university employs and from society, by filling pre-design Proforma with verbal and written consent taken from study participants and also explaining them about the purpose of the study. The duration of the study was six months (January 2019 to July 2019). Participants having the age of 30–70 years, normally healthy, obese and T2DM were included while T1Diabetic patients, Cardiovascular, hypertensive patients, pregnant ladies and patients who are on medication were excluded from the study.

2.2. Biochemical measurements

Present study distributed on two phases, in the 1st phase recruitment of participants by taking the history, anthropometric parameters and in 2nd phase 5 ml of blood was drawn by taking all aseptic measures then, 3 ml of blood was transferred into EDTA test tubes for genotyping and 2 ml of serum separating gel test tubes for fasting blood sugar (FBS), HbA1c, lipid profile. The blood was centrifuged for ten (10) minutes at 3000 rpm by centrifugation machine. The genomic DNA was extracted by Phenol-Chloroform Method (Ghatak et al., 2013), then resolved on 0.7% agarose gels electrophoresis, presence of single DNA in gel confirm the purity of DNA represented in (Fig. 1a). Specific primers were designed to amplify the DNA fragment containing rs-9939609 the variant of the *FTO* gene. The Forward and reserve primer sequence mention.

(5`-AACTGGCTCTTGAATGAAATAGGATTCAGA-3`)

(5`-AGAGTAACAGAGACTATCCAAGTGCAGTAC-3`)

PCR was used to amplify a particular DNA sequence, using PCR master mix, specific primers rs-9939609 of *FTO* gene with optimized PCR conditions. The amplified product was separated through agarose gel electrophoresis (1.2%) in TBE buffer as repre-

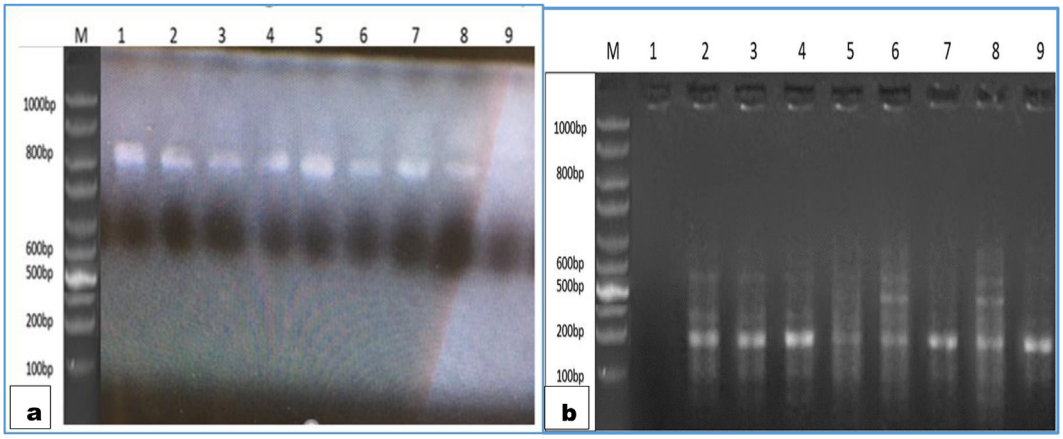


Fig. 1. (a) Genomic DNA extraction and resolved on 0.7% Agarose Gels Electrophoresis: Presence of Single DNA in Gel Confirm the Purity of DNA. (b) *FTO* Gene SNP rs-993906 variant PCR Amplified Products Represented from (lanes 1–9).

sented in Fig. 1b. The amplified PCR products were further confirmed by sequencing.

2.3. Statistical analyses

The data was entered on SPSS 23.0. then analysed the data by applying the ANOVA Post hoc (Tukey test), on both groups as compared to lipid profile, fasting blood sugar levels and HbA1c. To determine the allelic and genotypes frequency of *FTO* gene SNP variation in the study population. The data were analysed on the "SNP STAT" online website and analysed SNP frequencies by Hardy Weinberg Equilibrium (HWE). The p-values were derived by the chi-square test ($p < 0.05$).

3. Results

3.1. Demographic distribution of study participants

The demographic distribution of present study participants was graphically represented in Fig. 2. The age frequency distribution and mean age among study participants shown in Tables 1 & 2. The majority of the cases belong to the age group 40–49 years (56.3%) as shown in Table 1 respectively. The anthropometric characteristics of subjects were summarized in Table 2. The neck, waist, hip circumferences are considered as biomarkers of obesity and T2DM. The mean weight, BMI was increased and statistically significant difference of ($p < 0.001$), whereas the height of study participants was insignificant among groups. The mean blood pressure, temperature, fasting blood sugar and HbA1c among the study group was also insignificant as compared to the control represented in Table 2 respectively.

Table 1
Age frequencies of study participants.

Age (years)	Percentages (%)	Mean ± SD	P- value
30–39	(7.4%)	2.78 ± 1.47	<0.001
40–49	(56.3%)	3.37 ± 3.37	
50–59	(25.8%)	4.24 ± 1.66	
60–69	(8.4%)	3.56 ± 1.36	
Above 70	(2.1%)	5.0 ± 1.41	

3.2. Frequency distribution of BMI and lipid profile of study participants

The frequency of BMI among study participants represented in Fig. 3. According to WHO the weight classified as normal (18–24.9),

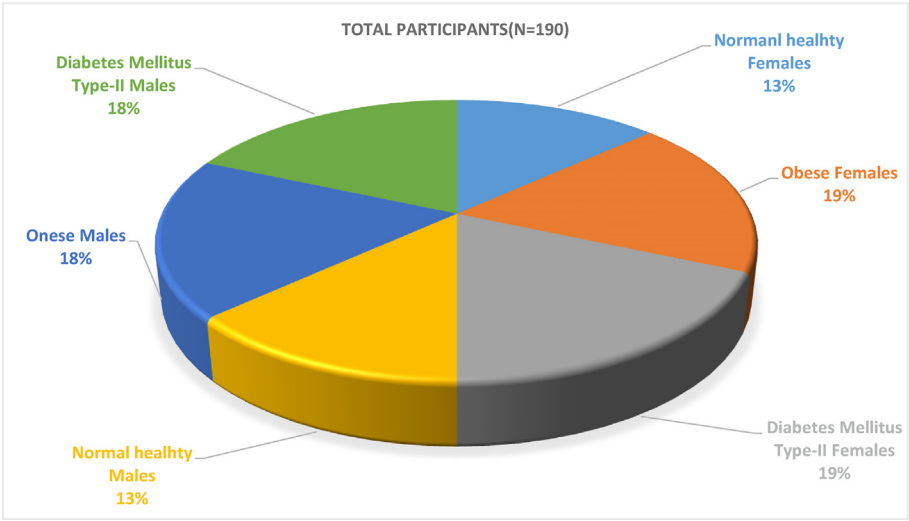


Fig. 2. Demographic distribution of study participants.

Table 2
Anthropometric Parameters of Obese and T2DM Participants as Compared with Normal Healthy Individuals.

Variables	Group-A Controls		Group –B Cases		Group- C Cases		p-value
	Healthy Females (n = 25)	Healthy Males (n = 25)	Obese Females (n = 35)	Obese Males (n = 35)	Diabetes Mellitus Type-II Females (n = 35)	Diabetes Mellitus Type-II Males (n = 35)	
Age (Years)	44.16 ± 4.29	45.92 ± 8.02	41.8 ± 11.3	44.6 ± 6.3	51.1 ± 6.9	53.1 ± 5.8	<0.001
Weight (Kg)	55.7 ± 7.9	62.8 ± 4.94	95.9 ± 11.2	102 ± 9.3	87.9 ± 11.5	99.5 ± 10.2	<0.001
Height (Cm)	160 ± 4.02	173 ± 4.8	162.5 ± 4.7	180.8 ± 6.01	158.8 ± 4.92	179.2 ± 6.12	NS = 0.006
BMI (Kg/m^2)	21.3 ± 2.0	21.0 ± 1.8	30.48 ± 3.9	32.5 ± 4.1	34.8 ± 4.9	31.8 ± 3.49	<0.001
Neck circumference (Cm)	32.8 ± 1.08	33.7 ± 1.8	39.9 ± 1.41	43.1 ± 2.01	39.3 ± 1.36	52.2 ± 2.3	<0.001
Waist circumference (Cm)	87.8 ± 0.31	98.1 ± 0.58	104.1 ± 2.0	122.8 ± 1.3	103.8 ± 2.2	107.2 ± 6.3	<0.001
Hip circumference (Cm)	88.7 ± 0.4	97.9 ± 102	105.4 ± 2.5	123.5 ± 1.46	104.9 ± 2.1	107.6 ± 6.4	<0.001
Waist/Hip ratio	0.98 ± 0.005	0.99 ± 0.01	0.99 ± 0.01	0.99 ± 0.005	0.99 ± 0.008	0.92 ± 0.05	=0.059 NS
Systolic Blood pressure (mmHg)	118 ± 13.5	120 ± 12.6	123 ± 6.9	125.6 ± 10.4	123.7 ± 9.5	134 ± 11.9	=0.053 NS
Diastolic Blood Pressure (mmHg)	72.7 ± 8.3	72.96 ± 7.9	78.6 ± 11.04	78.5 ± 12.6	83.8 ± 10.4	90.9 ± 11.03	=0.051 NS
Pulse (b/min)	72 ± 0.5	72.5 ± 0.1	72.1 ± 0.2	73.2 ± 0.1	73.5 ± 0.1	72.5 ± 0.1	=0.058 NS
Temperature (°F)	98.5 ± 1.5	98.6 ± 0.5	98.16 ± 1.2	98.5 ± 0.6	97.95 ± 1.1	98.9 ± 0.5	=0.056 NS
Fasting Blood Sugar (mg/dl)	83.76 ± 6.07	89.2 ± 6.4	95.42 ± 6.6	90.8 ± 16.3	129.7 ± 18.7	143.8 ± 34.54	<0.001
HbA1c (%)	4.84 ± 0.36	4.79 ± 0.39	5.36 ± 1.67	6.86 ± 0.84	8.15 ± 1.9	8.57 ± 1.61	<0.001

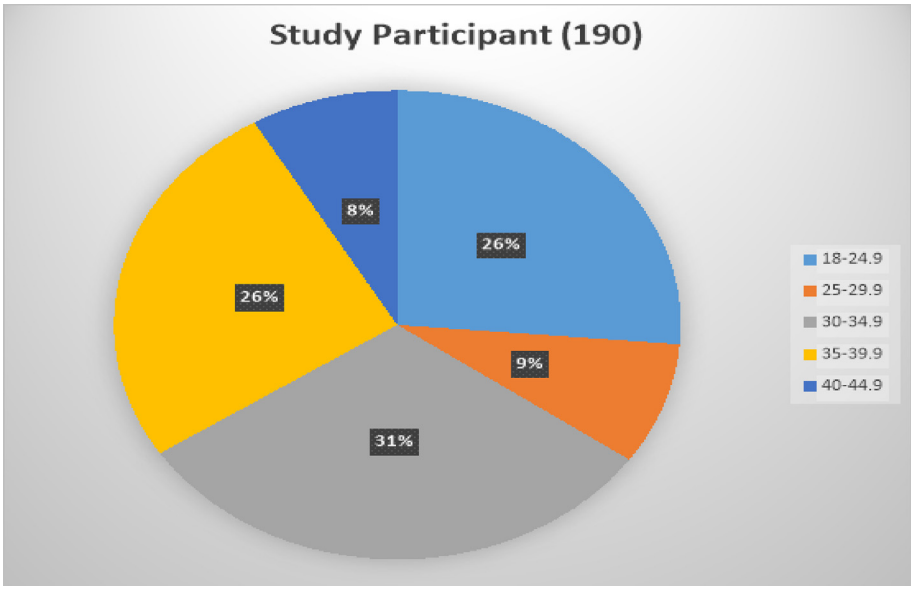


Fig. 3. Distribution of BMI(Kg/m^2) frequencies among study participants (n = 190).

overweight and obese, which is further categorized into Obese-I, II and III. The frequency of overweight was 31% among study groups, whereas 26% and 8% were obese class. The serum total cholesterol, TAGs, and VLDL-C of Type 2 diabetic and obese patients were more likely to high as compared to normal healthy individuals with a significant difference of ($p < 0.001$). whereas LDL-C was increased among the study group as compared to normal individuals of study groups, while (HDL-C) was decreased in T2DM and Obese as compared to control with a significant difference of ($p < 0.001$) shown in Table 3 respectively

Table 3
Comparison of Lipid Profile of Obese and Type 2 Diabetic participants among Normal Healthy Females by applying ANOVA-Test.

Variables	Group-A Controls		Group –B Cases		Group- C Cases		p-value
	Healthy Females (n = 25)	Healthy Males (n = 25)	Obese Females (n = 35)	Obese Males (n = 35)	Diabetes Mellitus Type-II Females (n = 35)	Diabetes Mellitus Type-II Males (n = 35)	
Total cholesterol (mg/dl)	135.8 ± 29.7	146.8 ± 22.7	235.8 ± 29.7	254.3 ± 46.4	296.9 ± 54.6	316 ± 66.1	<0.001
Triglyceride (mg/dl)	112.6 ± 19.9	132.7 ± 32.8	225.9 ± 129	149.2 ± 49.8	292.7 ± 49.8	223 ± 79.8	<0.001
HDL-C (mg/dl))	39.8 ± 6.98	40.5 ± 9.8	31.9 ± 6.83	35.6 ± 7.6	30.2 ± 7.6	33.9 ± 6.6	<0.001
LDL-C (mg/dl)	81.36 ± 18.9	97.32 ± 29.4	99.5 ± 36.8	114.2 ± 44.9	132.5 ± 36.7	147.7 ± 41.9	<0.001
VLDL-C (mg/dl)	24.8 ± 5.86	21.6 ± 7.5	26.4 ± 7.2	31.1 ± 22.6	32.9 ± 8.01	42 ± 14.9	<0.001

3.3. Distribution of genotypic and allelic FTO gene SNP rs-9939609 variant frequencies among healthy, obese and T2Diabetic study participants

FTO gene SNP rs-9939609 was found predominantly in the Hyderabad population of Sindh. The results of sequencing are shown in Figs. 4a, b & c. FTO gene SNP found in heterozygous TA genotypic variation, (SNP appeared A instead of T) represented in Fig. 4b. The distribution of allelic frequencies of study participants was more found in T/T "wild Type" 57.4%, T/A 37.9%, and very less AA (risk allele) 4.7% represented in Fig. 5 respectively. The *FTO* gene SNP-rs9939606 distributed in different age groups, the distribution frequencies are represented in Fig. 6. The frequency of the *FTO* gene SNP rs-9939609 variant was strongly associated with BMI, which more reliable biomarker of obesity and T2DM. summarized in Fig. 7 respectively. The genotype distribution of SNP rs-9939609 showed an increased risk of developing T2DM subjects, T/A showed a very strong association in codominant model OR 2.42, CI (1.23–3.84), p < 0.032, shown in Table 4.

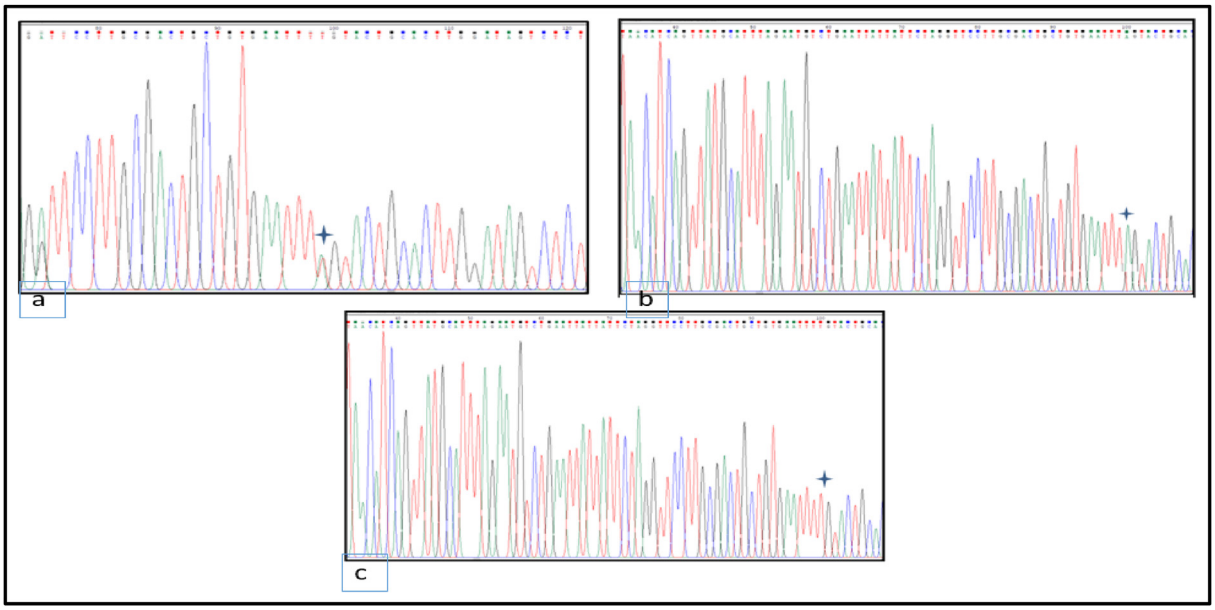
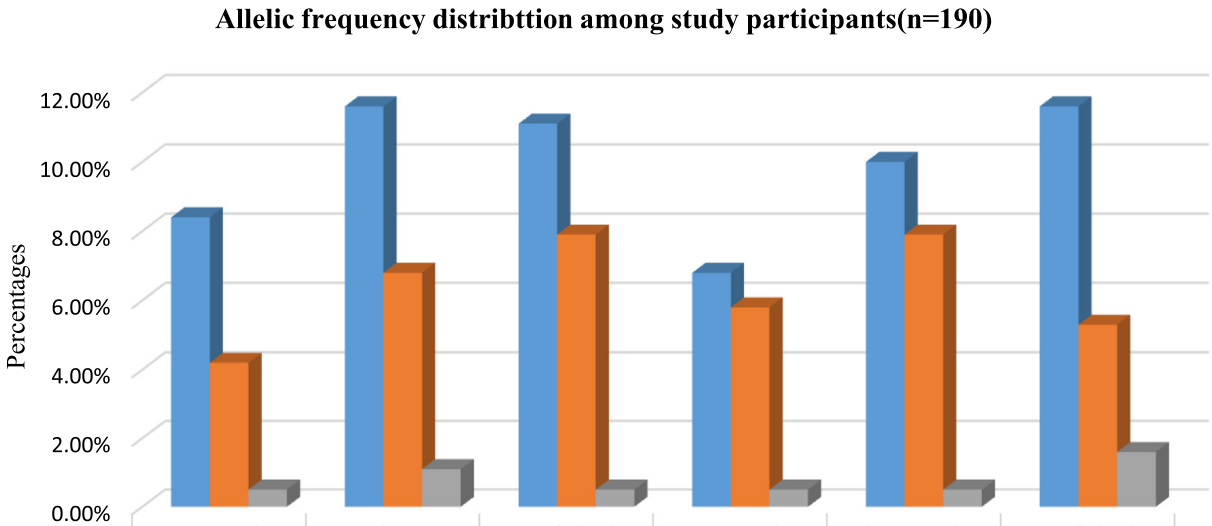


Fig. 4. (a) Chromatograph representing the FTO gene polymorphism in rs9939609 at 100 bp position SNP (T/A). (b) Chromatograph representing the FTO gene polymorphism in rs9939609 at 100 bp position SNP (T/T). (c) Chromatograph representing the FTO gene polymorphism in rs9939609 at 100 bp position SNP (T/A).



	Normal Healthy Females	Obese Females	T2Diabetic females	Normal Healthy Males	Obese Males	T2Diabetic Males
TT	8.40%	11.60%	11.10%	6.80%	10%	11.60%
TA	4.20%	6.80%	7.90%	5.80%	7.90%	5.30%
AA	0.50%	1.10%	0.50%	0.50%	0.50%	1.60%

Fig. 5. Allelic/genotypic frequency distribution of Study participants.

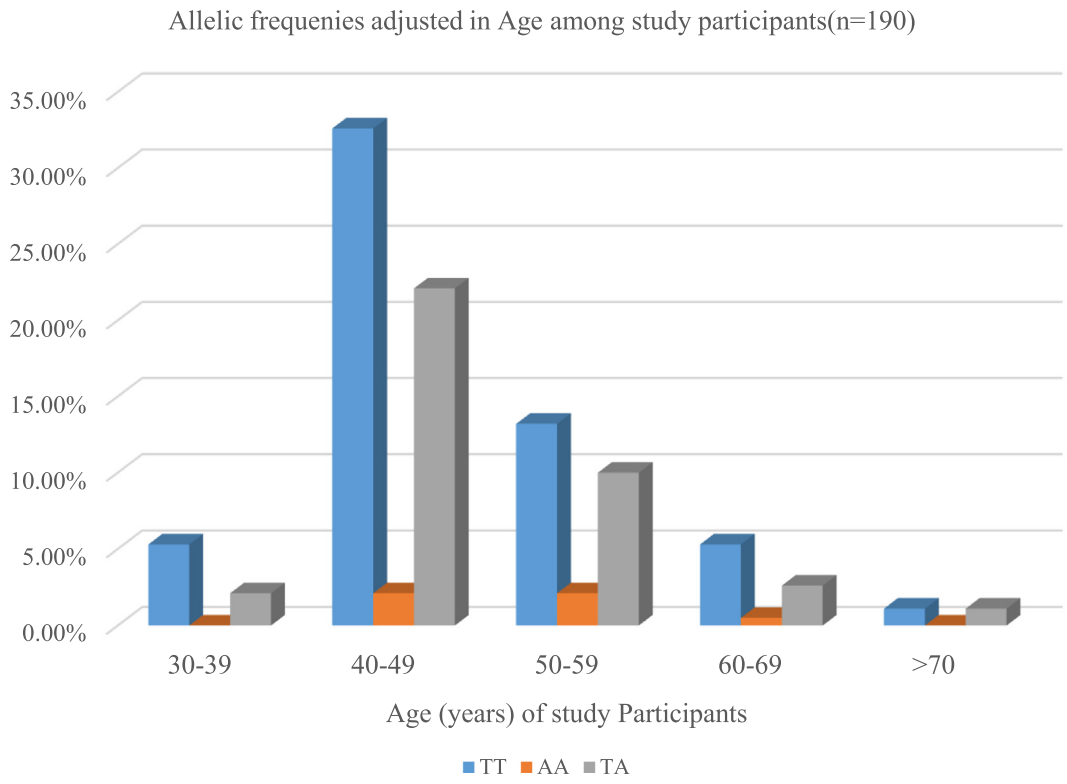


Fig. 6. Allelic Frequency distribution of FTO SNP-rs9939609 variant adjusted different Age groups among study participants.

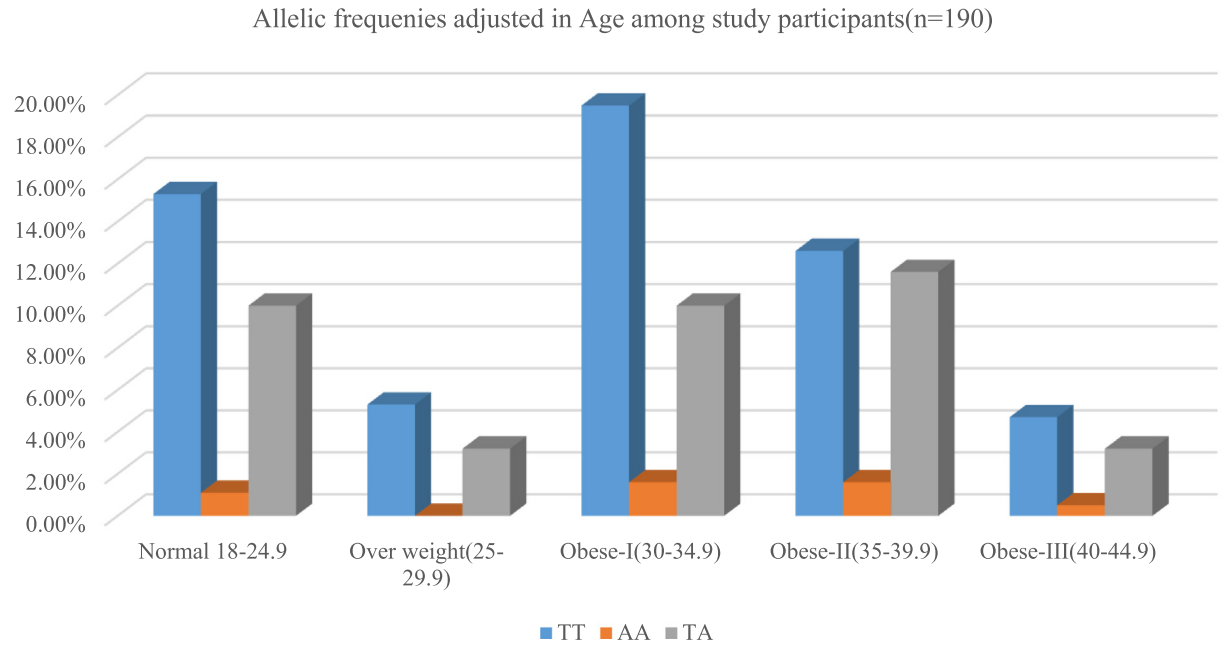


Fig. 7. Allelic Frequency distribution of FTO SNP-rs9939609 variant adjusted-BMI among study Groups.

4. Discussion

The FTO gene SNP rs-9939609 variant was found study population and showing strong association with T2DM. Vasudevan et al. 2010, reported that T2DM and obesity are rapidly growing health problem in Asia. Obese males and females are more prone to develop T2DM. In our study we also found the higher BMI in both males and females. Liu et al., 2011 revealed that basal metabolic index and waist hip ratio are the predictor of obesity and other metabolic syndromes. A retrospective study by Aravinda (2019) revealed that it is fact about obesity that obese were develop T2DM eight time more than non-obese. Scientific evidence also indicated that "waist circumference" is an earlier marker of T2DM than other anthropometric parameters, supporting present study results. Wang et al., 2016 considered that BMI, WC and WHR can be used as best predictors of T2DM, present study results

Table 4
FTO rs-9939609 SNP Association with BMI of Study Participants of Hyderabad analysed by Hardy Weinberg Equilibrium test (n = 190).

Model	Genotype	Cases (n=%)	Control (n=%)	OR(95 %CI)	p-value	AIC
Codominant	T/T	80(45.3)	35(15.2)	1.00	0.032*	784.5
	T/A	54(27.9)	19(10.0)	2.42(1.23–3.84)		
	A/A	7(3.7)	2(1.0%)	1.26(1.18–1.71)		
Dominant	T/T	80(45.3)	35(15.2)	1.00	0.05*	782.9
	T/A-A/A	61(31.6)	21(11.0)	1.23(2.75–1.38)		
Recessive	T/T-T/A	134(73.2)	54(25.2)	1.00	0.37	783.5
	A/A	7(3.7)	2(1.0)	0.58(1.83–0.67)		
Over dominant	T/T-A/A	87(49)	41(16.2)	1.00	0.012*	782.6
	T/A	54(27.96)	19(10.0)	2.62(1.45–4.87)		
Log-additive	–	–	–	0.41(0.86–0.39)	0.074	780

consistent with previous studies, except WHR. In present study we found insignificant WHR results. Waist circumference is a simple measurement of abdominal obesity and also useful marker of T2DM. Ahmad et al. (2016), revealed that waist circumference is best maker for early detection of T2DMand obesity, our results found to be similar. Alzeidan et al., 2019, revealed that neck circumference for females and males have independently associated with central and general obesity whereas, Ting et al., 2018, reported that upper-body subcutaneous fat measurement and neck circumference has been associated with central adiposity. Our study results were consistence with above said study, the neck circumference was found highly significant with difference of ($p < 0.001$). The lipid profile was found to be an important biomarker of T2DM and other metabolic syndromes. Naqvi *et al*., (Naqvi et al., 2017) reported that increased Triglycerides, and LDL related to insulin resistance results in T2DM and cardiovascular diseases. We found similar results in obese and T2DM.

Yang et al., 2017, observed that SNP rs9939609 was associated with higher BMI and obese men and females are at higher risk. We observed associations between *FTO* gene in adults age group and was found similar results, as reported previously.

Sabarneh et al., 2018, shown that *FTO* gene SNP having relationship with risk allele A/A but in our study AA found very fewer, instead of this we found significant T/A SNP frequencies. Younus et al. 2017, observed two different FTO gene SNP r-s17817449 and rs-9939609 and found significant T/A and A/A allele in rs-9939609 variant with increased risk for the development of T2DM in Iraqi individual. But we observed only one SNP with significant results. Moghanloo et al., 2018, reported mutant genotypes A/A and T/A in diabetic subjects having associated with increased total cholesterol, our finding was consistent with these results.

4.1. Conclusion

FTO gene SNP rs-9939609 variant was present in Hyderabad population of Sindh. BMI, neck, waist circumference strongly associated with FTO gene SNP. lipid profile also disturbed in study participants. Early detection of FTO gene SNP rs-9939609 variant might be help in prevention of T2DM by change the life style and exercise.

5. Recommendation

Further studies should be carried out on other SNPs of FTO gene and present SNP should be analysis in all districts of Sindh, Pakistan.

6. Ethical Consideration

The study was conducted strictly under the ethical rules after the approval from Ethical Review Committee of IBGE, University of Sindh, Jamshoro.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Further Reading

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