Original Article

Exposure of Fat Mass Obesity Gene Polymorphism in Diabetes Mellitus Type-II Females of Hyderabad, Sindh

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ABSTRACT

OBJECTIVES: To analyse and compare fat mass obesity gene polymorphism from blood samples in females with T2DM and Non-diabetic healthy females in population of Hyderabad, Sindh.

METHODOLOGY: A cross sectional comparative study was conducted at Institute of Biotechnology and Genetic Engineering University of Sindh, Jamshoro. The patients were recruited from Department of Medicine unit-II (LUH) Jamshoro/Hyderabad while non-Diabetic healthy females were recruited from society by filling Performa. The verbal and written consent was taken from all participants and explaining them about purpose of study. Finally blood sample for gene analysis and other biochemical tests were collected and stored in the laboratory at -20C. Total 100 females were included in this study out of which 50 were T2DM females and 50 were normal healthy females.

RESULTS: FTO (fat mass obesity) gene has strong association with basal metabolic index (BMI), as BMI increases the risk of T2DM in females also increases. We observed minor allele A and T at rs9939609 in Type 2 diabetic females with significantly higher with difference of (p <0.05) as compared with control group. In the present study rs9939609 FTO variant is strongly associated with Type 2Diabeticfemales of Hyderabad, Sindh.

CONCLUSION: In the present study rs9939609 FTO variant was strongly associated with Type 2 Diabetic females of Hyderabad, Sindh. The study was also aimed to aware the population about inherited FTO gene variant which might be possible to implement genetic screening of females with T2DM which will be help in prevention of T2DM in future.

KEY WORDS: FTO gene, T2DM, BMI, ARMS PCR, Electrophoresis

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INTRODUCTION

Diabetes Mellitus (DM) is heterogeneous and complex disease which causing health problem globally[1]. More than 300 million people live with diabetes worldwide[2]. Type 2 diabetes is characterized by persistent hyperglycemia due to dysfunction of pancreatic β-cell. Diabetes mellitus Type 2 (T2DM) has been shown to be a partially inheritable disease, in which genetic factor plays a significant role in the etiologic of the disease[3]. T2DM is more common type of diabetes Mellitus. Single nucleotide polymorphism (SNP) in Fat mass and obesity gene (FTO) are strongly associated with T2DM[4]. The prevalence of T2DM in Sindh province of Pakistan is 16.2% in males and 11.70 % in females[5].

FTO gene has nine exons and many single-nucleotide polymorphisms (SNPs)[6] which is located on chromosome 16 (16q12.2). According to genome-wide association study (GWAS) gene susceptibility of T2DM has common variant rs9939609 in the *FTO* gene that influences Asian populations for diabetes[7]. Nowadays researcher have focused on the correlation between *FTO* gene polymorphisms and T2DM in different populations[8]. The missense mutation in first intron of *FTO* gene at rs9939609 SNP with nucleotide position "53786615". It was found to that *FTO* gene have a strong association with BMI and T2DM in Asian[9].

Many SNPs of *FTO* gene were correlated with T2DM, but the results were different in different populations studied because of their lifestyle and food habits[10]. In human being the mRNA of *FTO* gene is expressed in a many tissues. *FTO* gene had first cloned and identified by fused-toe mutant mouse, whose phenotype results from a 1.6, Mb deletion of six genes along with *FTO*[11,12]. The *FTO* organized in double-stranded beta-helix fold (DSBH) which is associated with iron and 2-oxoglutarate oxygenases[13]. It is belonging to protein family known as AlkB and localize in the nucleus but mutation has not disturbed its nuclear localization[14]. The human and murine FTO have been exposed to in single strand of DNA and

RNA which are demethylate 3-methylthymine and 3-methyluracil residues in vitro however, FTO relatively less efficiency. In 2007 the FTO was considered as an obesity genebut SNP in FTO have a strong association with T2DM[15]. The aim of study was to aware the population about inherited *FTO* gene variant which might be susceptible for developing T2DM in females.

METHODOLOGY

This cross sectional comparative study was conducted at Institute of Biotechnology and Genetic Engineering University of Sindh, Jamshoro. The patients were recruited from Department of Medicine unit-II (LUH) Jamshoro/Hyderabad while non-Diabetic healthy females were recruited from society by filling proforma and written consent from all participants by explaining them about study during the period of January to July 2018.

The sample size calculated using the general formula of biostatistics for sample size calculation. Total 100 subjects were recruited and divided into two groups. Group A: n=50 Non-Diabetic healthy females as control, Group B: n= 50 Diabetes Mellitus Type-II females. Females with age of 40-60 years and Known Type 2 diabetic were included and exclusion of males, Type 1 Diabetic patients, hyperlipidemic, known cirrhotic patients, cardiac patient, hypertensive patients, pregnant ladies and patients who are on medication. The simple random sampling technique was used. All clinical data and relevant details of each healthy and T2DM females was registered by filling a proforma or Questionnaire. Verbal and written consent was taken from all participants by explaining them about the study purpose.

The study was comprised in two phases. In phase 1 recruitment of volunteers by taking history, anthropometric, BMI, fasting and random blood sugar and HbA1c and in phase 2 collecting blood sample from the all participants. Type 2 Diabetic females were recruited from Department of Medicine (LUH) Jamshoro/Hyderabad while Non-Diabetic healthy females were recruited from society. 5 ml of blood was drawn from each participant then 3 ml of blood was collected into EDTA test tubes for genotyping while and 2 ml of serum in gel test tubes for other biochemical tests. The blood was centrifuged at 3500 rpm for 10 minutes by centrifuge machine. The serum was fractionated and transferred to respected Eppendorf cups then stored at −20°C till required for analysis. Before the analysis sample was first allowed to attained room temperature then used. Various biochemical tests were performed such as fasting, Random blood sugar and HbA1c.

The chromosomal DNA was isolated through Phenol-Chloroform Method[16]. DNA was quantified spectrophotometrically and quality of DNA was observed through agarose(0.7%) gel electrophoresis. For amplification of *FTO* gene polymorphism, specific primers were designed to amplify the rs9939609 polymorphism at *FTO* gene. PCR was used to amplify a specific DNA sequence using PCR mixture, short oligonucleotides as primers specific rs9939609 region of *FTO* gene using Amplification Refractory Mutation System (ARMS PCR). Pair of outer and inner primers was designed for amplification of specific regions. PCR master mix contains PCR amplification buffer, dNTPs, Primers, $MgCl_2$, and genomic DNA with optimized PCR conditions[17]. The amplified DNA fragments were separated through electrophoresis agarose(1.2%) gels in TBE buffer.

Statistical analysis was performed on SPSS 16.0, Student t-test was applied on diabetic Type-II females as compared with healthy females. To determine the variation in distribution of alleles and genotypes within the concerned population by applying the chi square (x^2) and Hardy Weinberg Equilibrium test (HWE).

RESULTS

Total 100 females were included in this study. Anthropometric and metabolic characteristics of study population are summarized in Table I. The mean age, of control group was 40.2±5.7 years as compared to cases group 40.5±6.1 years which is not significant (NS=0.056). The mean fasting blood sugar of females with T2DM was high 140.4±12.5mg/dl as compared to healthy females were 90.5±6.8mg/dl. Random blood sugar of controls was 125±15.7mg/dl while as compared to cases group it was high 250±25.6mg/dl with highly significant difference of ($p<0.001$). The HbA1c of cases group was high 10.1±3.9% as compared to healthy controls 5.6±1.3% showing statistically significant($p<0.03$). The mean BMI of females with T2DM was increased 29.5±3.65 kg/m^2 as compared to controls was 23.4±0.27kg/m^2 with significant difference of ($p<0.001$) shown in Table I.

To determine the genotypes and allelic frequency variation of *FTO* gene within the concerned population were analysed by chi square (x^2) and deviation by Hardy Weinberg Equilibrium test are shown in Table II. The difference between frequencies for rs9939609 variant in both control and cases groups were highly significant (x^2/P/OR 9.85/0.01/3.7). FTO AA genotype frequency was less in female with T2DM as compared to healthy females. AT and TT genotype frequency was higher in females with T2DM as compared to healthy non diabetic females. While wild T and polymorphic A allelic frequency was (15.6/0.01/2.9) highly significant frequency of FTO genotypes was observed in T2DM cases as compared to the controls ($P<0.01$).

TABLE I: COMPARISON OF FEMALE'S AGE, FASTING, RANDOM BLOOD SUGAR, HBA1C, AND BMI BETWEEN CONTROLS AND CASES GROUPS OF TYPE 2 DIABETES MELLITUS BY APPLYING STUDENT T-TEST

Variable	Controls (n=50)	Cases (n=50)	p-value
	Mean and standard deviation		
Females Age (Years)	40.2±5.7	40.3±6.1	NS=0.056
Fasting blood sugar (mg/dl)	90.5±6.8	140.4±12.5	<0.001
Random blood sugar(mg/dl)	125±15.7	250±25.6	<0.001
HbA1c (%)	5.6±1.3	10.1±3.9	<0.03
BMI (Kg/m^2)	23.4±1.27	29.5±3.65	<0.001

TABLE II: GENOTYPE AND ALLELE DISTRIBUTION OF FTO GENE IN TYPE 2 DIABETIC FEMALES BY CHI SQUARE (X^2) & HARDY WEINBERG EQUILIBRIUM TEST

Genotype	Normal Healthy Females (n=50)	Females with Type 2 Diabetes Mellitus (n=50)	p value	x^2/P/OR
AA	11	3	<0.01	9.85/<0.01/3.7
AT	23	31	<0.03	
TT	16	19	<0.05	
ALLELES FREQUENCIES				
A	0.41	0.51	<0.01	15.6/<0.01/2.9
T	0.43	0.48	<0.01	

DISCUSSION

Type 2 diabetes mellitus is a complex disease with social and economic burden. Females who suffer from T2DM have a decreased life expectancy. Recent advances in genetic studies have contributed to identifications of susceptibility of gene polymorphism[18]. Polymorphisms within *FTO* gene strongly associated with T2DM. *FTO* gene has been widely explored because of its relationship with T2DM, obesity, cardiovascular diseases and metabolic syndrome.

Rees SD et al[19] reported in their study that *FTO* genotype and BMI are partly associated in Pakistani females, but in present study we observed that type 2 diabetics females of Hyderabad, having strong association of rs9939609 variant with *FTO* gene and BMI. This difference might be attributing to different ethnic groups, BMI standards sample size and environmental factors.

In a study from Asia[20] reported that as age increases the risk of diabetes in females also increases, the fasting and random blood sugar as well as high HbA1c levels in are higher in females which are consistent recent with our study results.

In European population[12] having high minor allele frequency (MAF) of 0.45 likewise other Asian studies[21] reported low minor allele frequencies. We also observed low frequency of minor allele in type 2 diabetic females as compared to healthy females. There are dissimilarities in MAFs in many of research studies, due to differences in BMI, ethnic groups and environmental factors. Despite low MAF in our study, we observed significant differences in genotype and allele frequency between normal healthy females and T2DM females of Hyderabad, Sindh.

In an investigation Younus LA 2017[23] also observed in Iraqi individuals that the presence of T allele in the two, SNPs rs9939609 and rs17817449 in the *FTO* gene polymorphisms which enhanced the risk of developing Diabetes mellitus Type II in their population. Aconsistent with our study results theminor allele (T) in rs9939609 was significantly higher (p <0.01) in T2DM females as compared with control group. Kaur R 2017[24] revealed that the association of *FTO* variant with T2DM were reported after age of forty. Similarly, in present study on females having age of more than forty showed association of allele A at rs9939609 with type 2 diabetes. In present study highly significant frequency of *FTO* genotype polymorphism was observed in females with T2DM compared with control group.

CONCLUSION

In the present study rs9939609 *FTO* variant was strongly associated with Type 2 Diabetic females of Hyderabad, Sindh. The study was also aimed to aware the population about inherited *FTO* gene variant which might be possible to implement genetic screening of females with T2DM which will be help in prevention of T2DM in future.

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