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ORIGINAL ARTICLE

Genetic Association of *FTO* rs9939609 Polymorphism with Hypertension in Pakistani Population

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ABSTRACT

Background: Hypertension is a medical condition that often occurs parallel to diabetes and obesity. Previously, the role of fat mass and obesity associated (*FTO*) gene rs9939609 polymorphism (c.46-23525T>A) with obesity has been reported while prevalence and etiological differences exist among different regions and ethnic groups.
Aim: To investigate the association of *FTO* rs9939609 SNP with hypertension in Pakistani population.
Study design: Cross-sectional study.
Place and duration of study: Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan from 1st January 2019 to 31st December 2020.
Methodology: One hundred and ten diagnosed hypertension patients along with 128 healthy volunteers were selected randomly. The single nucleotide polymorphism was analyzed using Amplified Refractory Mutation System-Polymerase Chain Reaction.
Results: In hypertension patients, females were found to be relatively more affected and average blood pressure lies in stage 1. However, we found no significant difference in genotypic frequencies of FTO rs9939609; TT (22.6%), AA (39.6%) and AT (37.8%) and TT (17.18%), AA (39.06%), and AT (43.75%) among HT and controls, respectively. The *p* and OR values for risk type genotype (AA) are 0.4697 and 0.7700 (95% CI=0.3788-1.565), respectively. Subsequently, the high percentage (60.0 %) of risk genotype (AA) was found in obese-body mass index in hypertension patients.
Conclusion: FTO rs9939609 single nucleotide polymorphism may not be a genetic risk factor associated with onset of hypertension directly and is linked with obesity in Pakistani patients.
Keywords: FTO, Hypertension, Obesity, Pakistani patients, rs9939609

INTRODUCTION

Hypertension is one of the most common non-communicable diseases that are associated with a variety of complications, including renal failure, ischemia heart disease and atheroma formation.[1] Global Burden of Disease study has reported HTN as the fourth contributor to premature birth defect deaths in developed countries and seventh in the developing countries.[2] Due to dwindling economic resources, the ratio of HTN treatment is relatively low in Pakistan[3]. The National Health Survey of Pakistan assessed that HTN influence 18% of grown-ups and 33% of individuals above 45 years of age[4].

Hypertension is a complex quantitative condition which is impacted by hereditary as well as environmental factors including anxiety, high salt uptake, and sedentary life style[4]. Primary HTN is idiopathic but many factors like age, gender, diabetes, obesity, smoking, diet, and medication have been associated with the secondary HTN.[5] So far many genes (mutations or SNP) are known to be associated with HTN, some of them are*CYP11B1*& 2, lysine-deficient protein kinase 1, 4 genes (*WNK1*, *WNK4*), kelch-like 3 gene (*KLHL318*), cullin 3 gene (*CUL318*), and potassium inwardly rectifying channel gene (*KCNJ5*).[6]

However, the fat mass and obesity-associated (*FTO*) gene is one of the hypertension susceptibility genes.[7] The cytogenetic location of *FTO* is on long arm of chromosome 16 (16q12.2) and encodes 2-oxoglutarate-dependent nucleic-acid demethylase, that is expressed in the hypothalamus and may have strong association with blood pressure.[8] Subsequently, the mutations or malfunctioning of *FTO* have been reported for onset of obesity and obesity related metabolic variations.[9-10] The SNP rs9939609is an intronicmissense variant (c.46-23525T>A) of *FTO* and most studied SNP in different populations in association with distinctive phenotypes[11-15]. Previously FTO polymorphism (rs9939609) has been found to be associated with coronary artery disease in Pakistani population[14] nevertheless the results are debatable and need more academic clarity. Some recent studies have also revealed its association with HTN in different population.[16-19] So keeping this in view, it is the need of time to unveil its association with HTN in Pakistani population.

Thus, the aim of this study was to investigate that either *FTO* (rs9939609) genotype were directly involved in the onset of hypertension in Pakistani population (male/female) by using ARMS-PCR method.

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MATERIALS AND METHODS

The study was approved by ethical committee and institutional review board of the International Islamic University, Islamabad, Pakistan. Informed written consent forms were obtained from all the participants of the study. The sample collection and experimental procedures were performed in accordance with the ethical standards of the Helsinki declaration 1964 and its latest amendments and permission from IRB.

One hundred and ten patients diagnosed HTN were enrolled with the medical history of diabetes. Hypertension diagnostic method which was used to calculate the blood pressure (BP) of HTN patients was sphygmomanometer. By this method systolic and diastolic blood pressure of the HTN patients were recorded after resting for 30 minutes. On the other hand controls (n=128) were selected under a strict criteria which include no history of HTN and its related drugs along with no history of diabetes. In both groups, female has major population. Both HTN diagnosed and control subject were over 30 years of age were recruited from Tertiary Care Hospital, Islamabad, Pakistan and research was conducted at the Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan. The inclusion criteria includes 1) studies which are assessing between FTO rs9939609 gene polymorphisms with hypertension. 2) Studies with case control and cohort design. 3) Studies conducted on HTN irrespective of gender. 4) Studies which provided enough data for estimating an odd ratios (OR) with 95% confidence interval. The exclusion

criteria includes 1) medication for losing or gaining weight. 2) Pregnancy or lactation. 3) Mental illness. 4) Martial status. 5) Cigarette smoker.

Blood samples were collected and centrifuge to obtain plasma/serum and stored at -80 degree. Biochemical parameters including sugar level (random and fasting sugar), creatinine and serum cholesterol. Triglycerides were calculated by commercially available enzymatic kit (ERBA Germany). Low density lipo-protein (LDL), high density lipo-protein (HDL)was calculated by Friedewald equation: LDL-cholesterol = total cholesterol – HDL cholesterol – triglyceride/5 (mg/dL). Other clinical parameters like age, gender, uric acid, urea, bilirubin, alkaline phosphatase (ALP) and family history were collected from patient's medical record folders.

The venous blood (5mL) was drawn from each participant and collected in Acid Citrate Dextrose (ACD) vacutainer (BD Franklin, Lakes NJ USA), which contained 2.5mL acid-citrate-dextrose solution. The vacutainer tubes were inverted few times gently and stored at 4℃ till further processing.

The genomic DNA (gDNA) from all of the samples was extracted using standard organic (Phenol-Chloroform) method as described previously[20]. In order to determine rs9939609A/T polymorphism in the *FTO*, ARMS-PCR was performed. Primer was designed by using Primer 1 software – Primer Designed web services for Tetra Primer, ARMS-PCR. The primers were designed by using Primer3 Input v.0.4.0 with default parameters. The primers sequences were; Forward Inner (5-TAGGTTCCTTGCGACTGCTGTGAATATA3), Reverse Inner (5-GAGTAACAGAGACTATCCAAGTGCATCTCA 3), Forward Outer (5-GTTCTACAGTTCCAGTCATTTTTGACAGC 3), and Reverse Outer (5-AGCCTCTCTACCATCTTATGTCCAAACA 3).

The ARMS-PCR was performed in 96 wells thermal cycler (Thermo Electron Corporation, Mill ford, USA). After optimization, all samples were amplified. The master mix (DNA solution, 10x PCR buffer, d.H20, MgCl2, 5U/µl Taq DNA polymerase) for all ARMS-PCR reactions with specific primer pair was prepared. The PCR reaction mixture containing all ingredients along with four primers FI, FO, RI, and RO spin for few seconds. The Tetra ARMS-PCR products were checked on 2.5% agarose gel containing ethidium bromide.

A comparison of normal weight patients was made between overweight and obese HTN patients. Mean age, BMI, BP systolic, BP diastolic, uric acid, urea, triglycerides, random sugar, fasting sugar, creatinine, serum cholesterol, AST, ALT, LDL, HDL, bilirubin, Alk-phos and frequency of gender (male/female) and family history were calculated by using statistical software SPSS 20.0.

To find the association of the FTO rs9939609 genotype and allele with the hypertension the p value and Chi square test was performed by using statistical program SPSS-20. A p-value <0.05 was considered as statistically significant.

RESULTS

The mean values and frequencies of different anthropometric parameters including age, gender, BMI, family history, BP (systolic and diastolic), blood sugar level (fasting and random), uric acid, urea, creatinine, LDL, HDL, AST, ALT, triglycerides, serum cholesterol bilirubin and ALP were calculated. Overall, the mean ages of patients were 49 years. However, females were significantly more affected (65.1%) than males (34.9%) with HT. The mean systolic (148.12±20.795) and diastolic blood pressure (95.69±9.293) of the studied population lies within the stage 1 (Table 1).

In order to investigate the polymorphism of *FTO* rs9939609, ARMS-PCR was used for genotyping and resulting bands were analyzed (Fig. 1). All the genotypes of the patients and controls were in Hardy-Weinberg equilibrium *p=0.0283* and *p=0.3911* respectively. The homozygous AA which is the major allele, show no gross variation between two groups as in hypertension patients 22.6% as compared to the controls 17.18% along with their Chi-square value 0.738 (p>0.05). Although the percentage is slightly higher but these results are not statistically significant as *p*=0.390 (*p*>0.05). The heterozygous AT genotype also show significant difference between controls and hypertension patients i.e. 43.75% and 37.8% and chi-square value of AT genotype of both groups was 0.434. However this difference was also insignificant *p*=0.510 (*p*>0.05). However the homozygous TT which is the minor allele was almost equal in both patients and controls i.e. 39.6% and 39.06% and their chi-square value was 0.004 (p<0.05) but this is also statistically insignificant results p=0.949 (*p*>0.05). The odd ratio value of all three genotype AA, AT and TT with 95 % CI (confidence interval) were 1.4584 (0.7246 to 2.9351), 0.7801 (0.4435 to 1.3722) and 1.0427 (0.5914 to 1.8384) respectively with the *p*=0.05 (Table 3). Odd ratios results also suggesting that the comparison of three FTO rs9939609 genotype between controls and patients were not significant because the CI does include 1 in their interval. As we compare the controls and hypertensive patients for the risk allele genotype TT (minor allele), it shows not a significant difference. Since FTO is obesity associated gene, results shows that it is almost equally distributed among controls and hypertensive patients. There were many studies conducted which shows not significant association of FTO gene (rs9939609) with hypertension. The genotypic frequencies were not significant and showing no association of rs9939609polymorphism with HTN in Pakistani patients. However, the percentage of obese-BMI HTN patients (60%) carrying the AA genotype was larger as compared to other categories of BMI HTN patients, thus indicating that the rs9939609 is not directly linked with HTN, but may occur simultaneously with obesity in Pakistani obese-BMI HTN patients.

Table 1: Clinical and anthropometric parameters of hypertension patients

Variable			**Mean±SD**	
Age (years)		Male	54.16±13.51	49.15±13.359
		Female	47.00±12.65	
BMI (kg/m2)		Male	26.45±4.63	28.16±5.88
		Female	29.24±6.36	
Gender		Male	34.9%	
		Female	65.1%	
Family history		Positive	44.3%	
		Negative	55.7%	
BP (mmHg)	Systolic	Male	148.30±23.18	148.12±20.795
		Female	147.85±18.99	
	Diastolic	Male	96.63±11.81	95.69±9,293
		Female	95.61±7.96	
Blood sugar (mg/dL)	Fasting	Male	122.71±43.98	110.86±40.82
		Female	106.77±40.16	
	Random	Male	145.20±49.22	136.97±53.97
		Female	133.92±56.20	
Uric Acid (µmol/l))				5.34±1.413
Mean Urea (mg/dL)				32.11±7.976
Creatinine (mg/dL)				1.43±1.960
LDL (mg/dL)				139.80±34.544
HDL (mg/dL)				40.50±2.506
Triglycerides (mg/dL)				270.22±193.787
Serum cholesterol (mg/dL)				220.57±57.374
AST (Unites per lit)				35.00±6,218
ALT (united per lit)				36.68±22.373
Bilirubin (mg/dL)				0.73±0.189
Alkaline phosphatase (IU/L)				223.56 ± 19.481

SD=Standard deviation, BMI=Body mass index, LDL=Low density lipoprotein, HDL=High density lipoprotein, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase

Table 2: Genotype and allele distribution of rs9939609 polymorphism in HTN patients and control group

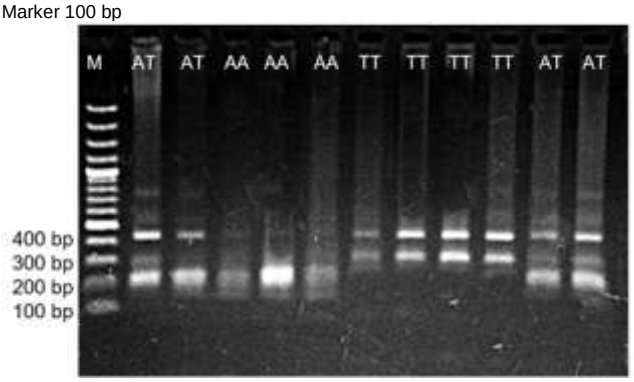
Group studied	rs9939609 Genotype (Ancestral: A)			Allele Frequency	
	AA	TT	AT	A	T
HTNH (106)	24 (22.6%)	42 (39.6%)	40 (37.8%)	0.415	0.585
H-W expected value p=0.0283	16.74	33.74	47.53	0.412	0..588
Control (N=128)	22 (17.18%)	50 (39.06%)	56 (43.75%)	0.390	0.61
H-W expected value (p=0.3911)	14.97	36.97	47.06	0.388	0.611
p-value	0.390	0.949	0.510	0.974	0.974
Chi-square value (CI 95%, DOF 1)	0.738	0.004	0.434	0.001	0.001
OR	1.4584 (0.7246-2.9351)	1.0427 (0.5914-1.8384)	0.7801 0.4435-1.3722)	1.1326 (0.6438-1.9927)	0.92 (0.5224-1.6203)

Table 3: Genotype and Allele percentages of rs9939609 among normal, overweight & obese HTN patents vs normal controls

Variable	Controls	Normal wt	Overweight	Obese
Genotype				
TT	22(17.18%)	8 (29.6%)	9(25.7%)	5(20.0%)
AA	50(39.06 %)	10(37.0%)	11(31.4%)	15(60.0%)
AT	56(43.75)	9(33.3%)	15(42.9%)	5(20.0%)
Allele				
A	0.39 %	0.463 %	0.471 %	0.3 %
T	0.61 %	0.54 %	0.53%	0.7 %

*Chi-square *p*<0.05, 95% CI=Confidence Interval, Odd Ratio (OR)>1, H-W= Hardy-Weinberg, HTN=Hypertensive patients, Ref= Reference genotype

Fig. 1: A 2% agarose gel electrophoresis of *FTO* rs9939609 in HTN Pakistani patients. The PCR product representing different genotypes. AA genotype (201 bp), AT genotype (436 bp) and TT genotype (293 bp). M= Marker 100 bp



DISCUSSION

Hypertension is a common medical condition which is distressing human population worldwide. Its prevention and treatment can be helpful for well-being of mankind[2].Current advancements have improved the understanding for pathophysiology of high blood pressure and therefore provides the novel therapeutic options to prevent HTN related complications, still it needs more improvement to control the early onset of HTN[2,21] Hypertension can be caused by multiple genetic and acquired risk factors[22] However, gender and age plays an important role in onset of many disorders. But, there is no obvious gender contrast in the pathophysiology of HTN[5,23]. In this study, the females had high risk of getting HTN as compared to males. The mean age of the selected HTN patients was 49±13years that is in accordance to the recent reported studies in which the blood pressure changes are more prevalent in age above than 45 years[5,23,24]. The rise in blood pressure with age is due to the some major structural changes in arteries and it has been reported that systolic blood pressure increases continuously with age as compared to diastolic blood pressure[25].

The rs9939609 polymorphism islocated in intron 1 of *FTO* genomic location at 87653T (c. 46-23525T >A, https://www.ncbi.nlm.nih.gov/snp/rs9939609). *FTO* rs9939609 polymorphism has correlation with many diseases as reported earlier.[24-27] In a study conducted on genetic risk of coronary artery disease (CAD) in Pakistani population, rs993969 has displayed a reasonable association with CAD (p=0.009).[14] On the other hand, a meta-analysis research study showed, rs9939609 polymorphism has an association with very few types of cancer i.e. endometrial cancer (OR=1.07, 95% CI=1.00-1.14) and pancreatic cancer (OR=1.12, 95% CI=1.04-1.21). However, after adjustment of BMI, this polymorphism shows relationship with high cancer risk in East Asian population (OR=1.29, 95% CI=1.06-1.57) and African population (OR=1.21, 95% CI=1.06-1.38).[15] This polymorphism has also shown a relation with metabolic syndromes that are also linked with diabetes.[19,26,28] Many studies showed more remarkable association of rs9939609with obesity in different populations.[29-33] Previously, only few studies illustrated the noteworthy relation of HT with *FTO* rs9939609[8,34] The recent study conducted by Raja et al[34] in Pakistani HTN population reported that A allele of rs9939609 is strong predictor of HTN. However, contrary to these studies no direct association of HTN has been found in subjected HTN patients. Likewise, Sabarneh et al[27] conducted a research in Palestine population and reported that *FTO* rs9939609 genotype has no association with HTN, age or gender. Similarly, Marcadenti et al[18] has also shown a high association of *FTO* polymorphism with BMI and type 2 diabetes mellitus but nullified the relation of this SNP with HTN. Therefore, rs9939609 may not be stated to have a direct association with HTN as depicted from most of association studies.

We have categorized the HTN patients on basis of their BMI, as shown in Table 3 and 4, and calculated the genotypic and allelic percentages. In comparison of overweight and obese-BMI patients with Normal-BMI HTN patients, about 60 % of Obese-BMI HTN patients have risk genotype for HT (Table 3). Similarly, when all the categorical variables of HTN were compared with subjected controls (n=128), high percentage (60%) of risk genotype (AA) was found in obese-BMI HTN patients. These percentages show that the HTN patients that suffer with obesity may represent a role of rs9939609in association with HTN. These findings were in accordance to the previous and recent reported studied which concluded that rs9939609 may not be a genetic risk factor for onset of HTN but may occur rationally with obesity.[18,35] In support to these findings, McMurray and Coxby in vivo studies have confirmed *FTO* rs9939609 significant role in weight gain.[37] Our study has limitations of less sample size and sampling is not much random as samples are collected from a single center, due to logistics samples were not collected from all regions of Pakistan.

CONCLUSION

FTO rs9939609 variant is not directly associated with development of HTN in Pakistani population. However, the obese Pakistani patients carrying the AA genotype are most probably at high risk of developing HTN. We suggest to conducted similar study on larger scale (>1000 participants) to evaluate the role of *FTO* rs9939609 polymorphism in Pakistani HTN patients for associated morbidities.
Conflict of interest: Nil

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