RESEARCH ARTICLE

TCF7-L2 rs7903146 polymorphism in metabolic syndrome with and without acute coronary syndrome

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Abstract

Objectives: To determine the frequency and association of single nucleotide polymorphism of transcription cell factor7-like2 rs7903146 (C>T) in metabolic syndrome patients with and without acute coronary syndrome.

Methods: The cross-sectional comparative study was conducted at the University of Health Sciences, Lahore, Pakistan, from July to December 2017. Patients of metabolic syndrome with and without acute coronary syndrome were selected from Sheikh Zayed Hospital, Lahore, and the Punjab Institute of Cardiology, Lahore. Healthy subjects were enrolled to act as controls. A fasting blood sample of 8ml was taken for deoxyribonucleic acid extraction and estimation of biochemical parameters. Single nucleotide polymorphism of transcription cell factor7-like2 rs7903146 C>T was determined using restriction fragment length polymorphism. SPSS 22 was used for data analysis.

Results: Of the 500 subjects, 200(40%) were group A patients without acute coronary syndrome, 100(20%) were in group B with acute coronary syndrome and 200(40%) were group C controls. Overall, 385(77%) were males and 115(23%) were females. The frequency of CC variant in group A was 35(17.5%) and in group C 22(11%), while CT was 32(16%) and 65(32.5%), and TT was 133(66.5%) and 113(56.5%), respectively. There was significant association of TT genotype with increased risk of metabolic syndrome (p=0.031), and CC genotype had no association (p=0.121). There was no significant difference of genotype frequency between groups A and B (p=0.246), but TT variant was significantly higher in group A compared to group B (p=0.009).

Conclusion: TT genotype of transcription cell factor7-like2 rs7903146 C>T was found to be associated with increased risk of metabolic syndrome in patients without acute coronary syndrome compared to those with acute coronary syndrome and healthy controls.

Keywords: TCF7-L2, Single nucleotide polymorphism, Acute coronary syndrome, Metabolic syndrome, HOMA-IR. (JPMA 70: 1774; 2020) **DOI: http://doi.org/10.5455/JPMA.45480**

Introduction

Metabolic syndrome (MetS) is an emerging global health problem. It is a cluster of cardiometabolic derangements, such as insulin resistance (IR), central or perivisceral adiposity, hypertension (HTN) and atherogenic dyslipdemias. All these MetS-associated factors considerably increase the risk of type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD).[1] Transcription cell factor7-like2 (TCF7-L2) belongs to the family of TCF/lymphoid enhancer binding class of transcription factors and exhibits large variety of funtions within the cell. TCF7-L2 regulates gene expression by acting through Wnt-beta(?)catenin signalling pathway.[2] It represses the pro-glucagon gene expression in the enteroendocrine cells. Proglucagon produced by the enteroendocrine cells is modified post-translationally to glucagon-like-peptide1 (GLP-1), which is responsible for the increased production of insulin and decreased release of glucagon.[3,4] Gene for TCF7-L2 is located on chromosome 10q25.2 and its single nucleotide polymorphisms (SNPs) are reported to be involved in the pathogenesis of T2DM by decreasing insulin secretion.[5] Earlier studies suggest that genetic variants of TCF7-L2 are associated with T2DM by various mechanisms, such as altered release of incretins, decresaed insulin secretion and incresaed gluconeogenesis.[6,7] In a study on Scandinavians, CT/TT genotype of TCF7-L2 rs7903146 C>T was found to be associated with 5-fold release of TCF7-L2 in panreatic-β cells, decrease in insulin and an increase in glucagon levels.[7] The minor genotypes TT of the common variants of TCF7-L2 rs7903146 (C>T) and rs12255372 (G>T) were also observed to be associated with progression to T2DM in various cohort studies.[8-10] There are various studies from Pakistan and other regions reporting the association of TCF7-L2 rs7903146 with T2DM, but data is limited considering the association of this polymorphism with MetS and acute cornonary syndrome (ACS).[11-13] The current study was planned to determine the association of genetic variants of TCF7-L2 rs7903146 (C>T) with MetS with and without ACS.

Subjects and Methods

The cross-sectional comparative study was conducted at

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the University of Health Sciences (UHS), Lahore, Pakistan, from July to December 2017. MetS patients with and without ACS were selected from Sheikh Zayed Hospital (SZH), Lahore, and the Punjab Institute of Cardiology (PIC), Lahore. Healthy subjects were enrolled to act as controls. After approval from the three institutional review boards the sample size was calculated using the equation:[14]

$$n = \frac{\left(Z_{1-\alpha/2}\sqrt{2\bar{p}(1-\bar{p})} + Z_{1-\beta}\sqrt{p_1(1-p_1)p_2(1-p_2)}\right)^2}{(p_1-p_2)^2}$$

$$Where\ \bar{p} = \left(\frac{p_1+p_2}{2}\right)$$

The desired power of the study was 80%, level of significance 0.05, minor allele frequency 0.28 for cases and 0.14 for controls.[15] Recruited cases of MetS without ACS in group A were outpatients registered with the SZH Diabetic Clinic, while those with ACS were PIC inpatients. Group C had the controls. The cases were approached at the Diabetic Clinic and the Cardiology wards. After written ionformed consent, data was collected using a questionnaire that recorded demographics, disease history, medications, and physical and biochemical parameters. MetS was defined according to the International Diabetes Federation (IDF) criteria.[16] All the cases were centrally obese, i.e., males with waist circumference (WC) ≥90cm and females ≥80cm with any two of the following four features; serum triglycerides (TG) ≥150mg/dl or on treatment; serum high-density lipoprotein (HDL) <40mg/dl in men and 50mg/dl in women or on treatment for dyslipidemia; blood pressure (BP) >130/85 or on treatment for HTN; and fasting blood glucose (FBG) >100mg/dl or on treatment for DM. ACS included unstable angina, ST elevation and non-ST elevation myocardiac infarction (NSTMI). Diagnosis of ACS was established by the cardiologists based upon electrocardiography (ECG) findings and elevation of cardiac enzymes Troponin T, Troponin I and cardiac isozymes. Cases having evidence of end-stage renal disease (ESRD), hepatic decompensation, chronic infective and inflammatory states or secondary causes of DM were excluded. Controls mathced for age and gender were selected from the general population. Those enrolled were non-hypertensives and non-diabetic with WC ≤90cm in men and ≤80cm in females with no history lipid-lowering agents or any medications for DM and HTN.

WC and BP were recorded by standard methods. Fasting blood sample of 8ml was taken from the subjects after an overnight fast of 8-10 hours. Blood for deoxyribonucleic acid (DNA) extraction was stored at -20°C and serum was separated and stored at -80°C. FBG and lipid profile, including serum TG and HDL, were measured by colorimetric method (Randox Kits, United Kingdom). Insulin was measured by human insulin enzyme0linked immunosorbent assay (ELISA) kit (Elabscience, Germany). Insulin resistance was calculated by homeostatic model assessment for insulin resistance (HOMA-IR) using the equation:[17]

HOMA-IR = Fasting serum glucose x Fasting serum insulin / 22.5

SNP of TCF7-L2 rs7903146 (C>T) was genotyped by restriction fragment length polymorphism (RFLP) using the following primers; Forward5'-AAGAGAAGATTCCTTTTTAAATGGTG-3' and Reverse5'-CCTCATACGGCAATTAAATTATACA-3'.[18] Polymerase chain reaction (PCR) was carried out in the thermal cycler. Initial denaturation was carried at 95°C for 15 minutes, followed by 34 denaturation cycles at 95°C for 30 seconds, annealing for 30 seconds at 58°C, extension for 30 seconds at 72°C and a final extension for 9 minutes at 72°C. The amplicons were run for 45 minutes on 3% ethidium bromide stained agarose gel, and checked under ultraviolet (UV) gel documentation system (Bio Rad, United States) for 136-bp product. The amplified product was digested by CH4III (Hpy-CH4III) or Taa1 (10U/µL, Thermo Scientific) at 65°C for 16 hours. The reaction was set in a volume of 10µL containing the amplicons, 1µL of CH4III or Taa1 (10U/µL, Thermo Scientific), 2µL 10X Tango buffer and 18µL of nuclease-free water. The digested products were separated on 3% agarose gel and visualised under UV documentation system (Bio Rad, US). The product sizes were for CC 136-bp, TT 112-bp and 24-bp, and CT 136-bp, 112- bp and 24-bp products.[18]

Data was analysed using SPSS 22. Medians with interquartile range (IQR) were used to express quantitative variables as data was non-normally distributed ($p<0.05$ by Shapiro-Wilk's statistics). Frequencies and percentages were used for categorical variables. $P<0.05$ was considered statistically significant. Genotypic and allelic frequencies were calculated and Hardy-Weinberg equilibrium (HWE) was determined.[19] Allelic frequencies of two groups were compared by Soft-pad graphics. In order to study the association of genotypes with MetS, three genetic models co-dominant (TT, TC, CC), dominant (TT; TC+CC) and recessive (CC; TC+TT) were constructed. Genotype frequencies of the two case groups were compared by chi-square test and odds ratio (OR) was calculated. Logistic regression was applied to see the association of genotypes with MetS and ACS after controlling for age and gender.

Results

Of the 500 subjects, 200(40%) were group A cases without ACS, 100(20%) were in group B with ACS, and 200(40%) were group C controls. Overall, 385(77%) were males and 115(23%) were females. The mean age of the cases was 47.23±7.82 compared to 46.48±8.35 years for the controls (p=0.357). Among the cases 237(79%) were diabetics, 27(9%) had impaired FBG, 234(78%) had HTN history and were on anti-hypertensives, 279(93%) had dyslipidaemia and were on lipid-lowering agents. The frequency of CC variant of TCF7-L2 C>T in group A was 35(17.5%) and in controls 22(11%); CT was 32(16%) and 65(32.5%), and TT was 133(66.5%) and 113(56.5%), respectively. The genotype frequencies in cases and controls deviated from HWE (p>0.05). In the co-dominant model, there was significant difference of the genotypes of TCF7-L2 C>T between the cases and the controls (p<0.001). Frequencies of the minor 'C' allele in cases and controls were 102(26%) and 109(27%) and of the major/wild T allele 298(74%) and 291(73%), respectively (Table-1.). No significant allelic associations of TCF7-L2 C>T was found with the MetS (p= 0.571). In the dominant model, TT genotype was associated with increased risk of MetS (p=0.031), but in the recessive model, no significant association of CC genotype was found with MetS (p=0.121). In logistic regression, the value remained

Table-1: Comparison of transcription cell factor7-like2 (TCF7-L2) rs7903146 (C>T) genotypic and allelic frequencies between metabolic syndrome patients without acute coronary syndrome and the healthy controls.

Genotype	MetS without ACS n(%age)	Healthy group n(%age)	p- value	OR and CI
Co-dominant model				
CC	35(17.5)	22(11)	0.000*	OR not computed
TC	32(16)	65(32.5)		
TT	133(66.5)	113(56.5)		
Total	200	200		
Allelic model				
C	102(26)	109(27)	0.571	0.91(0.68-1.25)
T	298(74)	291(73)		
Total	400	400		
Dominant model				
TT	133(66.5)	113(56)	0.031*	1.56(1.04-2.34)
TC+CC	67(33.5)	87(44)		
Total	200	200		
Recessive model				
CC	35(17.5)	22(12)	0.121	1.56(0.89-2.73)
TC+TT	165(82.5)	178(88)		
Total	200	200		

A "Chi square test" was applied to calculate the "p" value, odds ratio (OR) and confidence interval (CI). A "p" of < 0.05 is statistically significant. Logistic regression was applied and adjusted p-value was calculated after controlling for age and sex for the Dominant model. The value remained significant (p=0.034: Exp(B)=1.55: CI=1.03-2.33).

Table-2: Comparison of transcription cell factor7-like2 (TCF7-L2) rs7903146 (C>T) genotypic and allelic frequencies between metabolic syndrome patients with acute coronary syndrome and the healthy controls.

Genotype	MetS with ACS n(%age)	Healthy group n(%age)	p-value	OR and CI
Co-dominant model				
CC	14(14)	22(11)	0.088	OR not computed
CT	35(35)	65(32.5)		
TT	51(51)	113(56.5)		
Total	100	200		
Allelic model				
C	63(31.5)	109(27)	0.278	1.22(0.84-1.78)
T	137(68.5)	291(73)		
Total	200	400		
Recessive model				
CC	14(14)	22(12)	0.337	1.39(0.71-2.77)
TC+TT	86(86)	178(88)		
Total	100	200		
Dominant model				
TT	51(51)	113(56)	0.246	1.34(0.81-2.19
TC+CC	49(69)	87(44)		
Total	100	200		

A "Chi square test" was applied to calculate the "p" value, odds ratio (OR) and confidence interval (CI). A "p" of < 0.05 is statistically significant.

Table-3: Comparison of transcription cell factor7-like2 (TCF7-L2) rs7903146 (C>T) genotypic and allelic frequencies between metabolic syndrome patients with and without acute coronary syndrome.

Genotype	MetS without ACS n(%age)	MetS with ACS n(%age)	p-value	OR and CI
Co-dominant model				
CC	35(17.5)	14(14)	0.001*	OR not computed
TC	32(16)	35(35)		
TT	133(66.5)	51(51)		
Total	200	100		
Allelic frequency				
C	102(26)	63(31.5)	0.124	1.34(0.92-1.95)
T	298(74)	137(68.5)		
Total	400	200		
Recessive model				
CC	35(17.5)	14(14)	0.744	1.11(0.58-2.13)
TC+TT	165(82.5)	86(86)		
	200	100		
Dominant model				
TT	133(66.5)	51(51)	0.009*	1.91(1.18-3.18)
TC+CC	67(33.5)	49(69)		
Total	200	100		

A "Chi square test" was applied to calculate the "p" value, odds ratio (OR) and confidence interval (CI). A "p" of < 0.05 is statistically significant. Logistic regression was applied and adjusted p-value was calculated for the Dominant model after controlling for age and sex. The value remained significant (p=0.009: Exp(B)=1.93: CI=1.03-2.33).

Table-4: Comparison of study parameters in different genotypes of transcription cell factor7-like2 (TCF7-L2) rs7903146 (C>T) in the dominant model.

Parameters	TT	TC+CC	p-value
Systolic BP in mm of mercury	125(110-130)	120(110-130)	0.728
Diastolic BP in mm of mercury	80(70-90)	80(70-85)	0.989
Waist circumference in cm	106(100-113)	100(90-106)	0.042*
Serum triglycerides in mg/dl	171(131-239)	164(136-243)	0.168
Serum HDL in mg/dl	39(34-44)	38(35-41)	0.973
Serum cholesterol in mg/dl	196(171-270)	191(170-218)	0.908
Serum glucose in mmol/L	7(5.4-9.7)	6.2(4.9-9.1)	0.158
Serum insulin in µIU/ml	20(11-38)	17(9-31)	0.094
HOMA-IR	7.4(2.9-15)	5.5(2.2-10)	0.025*

p -value is calculated by Mann-Whitney U test. A p of less than 0.05 is statistically significant*. BP: Blood pressure; HOMA-IR: Homeostatic model assessment for insulin resistance.

significant (p=0.034).

There was no significant difference in the genotype frequency of TCF7-L2 C>T variants between hroups B and C (p=0.246). However, the TT variant was significantly higher (Table-3) in group A cases compared to group B cases (p=0.009). In logistic regression, p-value remained significant (p=0.009).

WC and HOMA-IR were significantly higher in the TT genotype of TCF7-L2 compared to the TC+CC (p=0.042 and p=0.025), but there was no significant difference of the other anthropometric and metabolic parameters in the different genotypes of TCF7-L2 C>T (Table-4).

Discussion

In the current study, significant risk of MetS was found to be present in the TT genotype of TCF7-L2 rs7903146 C>T, while no association of this SNP was observed with ACS. These results are in concordance with other studies done on Pakistani population which also reported the association of mutant allele of TCF7-L2 C>T with increased risk of Type 2 Diabetes Mellitus (T2DM).[11,20] In one study, genetic variants CT and TT of rs7903146 C>T were found to confer the risk of T2DM in patients with impaired glucose tolerance (IGT) by the decline in pancreatic beta cell mass and a decrease in insulin secretion.[8] Minor alleles of the transcription factors TCF7-L2 rs12255372 G>T and rs7903146 C>T were found to be associated with the increased risk of T2DM in African Americans, non-Hispanic Whites and Han Chinese.[12,21] Also, T alleles of rs12255372 G>T and rs7903146 C>T showed evidence of association with the increased risk of T2DM.[22] T allele of rs12255372 G>T was associated with the significant risk of T2DM in US population in men and women.[23]

The frequency of the mutant T allele of TCF-7L2 rs7903146 C>T, in our study, turned to be 0.74 whereas frequency of the other C allele was 0.26. Frequency of the T allele in the current study was considerably higher compared to the value in a previous study from Pakistan which reported T allele frequency of 0.41 and 0.36 in cases and controls respectively.[11] In a study from Hyderabad, India, frequency of the T allele in cases and controls was 0.31 and 0.21, respectively.[13] Allelic frequencies of our study were comparable and in concordance with the findings of a previous study from Cameroon that reported the frequency of T allele to be 0.9 and 1.0 in cases and controls, respectively, and of C allele 0.1 in cases and none in the controls. However, C allele was associated with the increased risk of DM.[18] In our study, no association of TT genotype was found with ACS in MetS and this result was in concordance with the findings of a study from Brazil that also reported lack of association between TCF7-L2 C>T variant and coronary artery disease (CAD) in diabetics, but association of TT variant was found with CAD in non-diabetics.[24] WC and HOMA-IR were found to be significantly higher in the TT variant of TCF7-L2 compared to the TC+CC in the present study. MetS is a cluster of biochemical and clinical derangements resulting from intricate influence of genetic and environmental elements. Core defect in MetS and its associated traits, such as HTN, dyslipidaemia and altered glycaemic parameters, is insulin-resistance. Decreased response of the target areas, like liver, skeletal muscle and adipose organs, to insulin precede many years prior to the onset of beta cell dysfunction.[16] TCF7-L2 is reported to be involved in glucose metabolism through Wnt signalling pathway by regulating pancreatic beta cell growth during the embryonic life. Variants of TCF7-L2 predispose to an increased risk of T2DM and IR by decrease in insulin secretion.[25] However, in the present study, the intervening connection between the variant genotype and the phenotype is missing as we did not check the messenger ribonucleic acid (mRNA) of TCF7-L2 to see the effect of its C>T polymorphism on the gene expression.

Conclusion

TT genotype of TCF7-L2 rs7903146 C>T was found to be associated with the increased risk of MetS in subjects without ACS compared to the controls. Frequency of TT variant of TCF7-L2 was significantly higher in MetS patients without ACS compared to those with ACS. WC and HOMA-IR levels were found to be significantly higher in the TT variant of TCF-7L2 compared to the TC+CC.

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