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A preliminary study on the association of glutathione S- transferees (GSTM1 and GSTTI) gene polymorphisms with type-2 diabetes mellitus among Telangana population

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ABSTRACT

Aim: Genetic susceptibility plays an important role in the causation of type 2 diabetes mellitus. We have carried out a case control study to investigate the association of the polymorphisms of GSTM1 and GSTT1 with the risk of type 2 DM among Telangana population.
Methodology: Blood samples were collected from 30 patients with type 2 DM and equal number of healthy individuals and genotyping was done using multiplex polymerase chain reaction. Statistical analysis was performed by using Graphpad prism version 5 to understand the association of the polymorphisms of GSTM1 & GSTT1 genes with T2 DM.
Results: The GSTT1 null genotype was significantly associated with the risk of developing T2DM.No association was observed between the polymorphisms of GSTM1 and T2DM.
Conclusion: The preliminary results suggest that GSTT1 null genotype may contribute to the development of T2DM in Telangana population. However further studies in larger sample size are warranted to arrive at definitive conclusion.

Keywords : Type 2 diabetes mellitus, GSTM1 gene, GSTT1 gene, Telangana population.

INTRODUCTION

Diabetes mellitus is a global health concern characterized by impaired metabolism of glucose and lipids due to defects in insulin secretion (beta cell dysfunction) or action (insulin resistance) and can affect children, adolescents and adults when the pancreas does not produce enough insulin or the body cannot effectively use the insulin produced by the pancreas (Adina, 2015). Type 2 diabetes mellitus (T2DM) is the most common disease and a serious global health problem. T2DM develops through an exposure to environmental risk factors, lifestyle habits and genetic susceptibility and it is characterized by chronic hyperglycemia and other metabolic alterations (Denise et al.,2013). The etiology of T2DM is complex and is associated with irreversible risk factors such as age, race, and

ethnicity and reversible factors such as diet, physical activity, smoking and alcohol consumption (Waqas et al., 2017).

The 6th edition of IDF (International Diabetes Federation) Diabetes Atlas.,(2014), showed that 382 million people in the age group of 20-79 years have T2DM throughout the world. This is expected to increase by 55% in the year 2035 with the figure rising to 592 million. The most dangerous fact about this particular disease is that about 50% individuals are undiagnosed. The epidemiological survey also states that the South-East Asian countries had a diabetic population (20-79 years) of 72.1 million in 2013 with 49% undiagnosed diabetic subjects and it might increase to 71% i.e. 123 million by 2035 (IDF, 2014).

A number of genetic factors may be responsible for diabetes. It has also been reported that defects in antioxidant defense against oxidative stress play an important role in the etiology of diabetes and its complications (Opara.,2002, Friedly Philipson.,2005). Glutathione-S-transferases are phase II key detoxifying enzymes, which play an important role in cell protection against a wide variety of toxic insults caused by chemical products, metabolites, oxidative stress products, and electrophiles. They are involved in the conjugation with glutathione of a broad range of electrophilic substances, thus facilitating their detoxification, metabolism, and excretion (Amer et al.,2011). Generally genetic polymorphisms associated with low or altered enzyme activity are being reported for GSTM1 and GSTT1 (Gonul.,2012),influencing the clearance of the DNA toxic intermediates, being partially responsible for the individuals susceptibility of pancreatic beta cells and possibly the peripheral nerves to oxidative stress. The most important gene polymorphism encodes a partial deletion of the GSTM1 gene locus on chromosome 1p13.3 (GSTM1 null genotype) which causes complete absence of enzyme activity. GSTT1 gene, located on chromosome 22q11.2, presents a deletion similar to the GSTM1 polymorphism (Amer et al.,2012, Miller and Neuberg.,2003). Several studies reported significant association of T2DM with both null genotypes of GSTM1 and GSTT1, while some other studies showed no association between GSTT1 and GSTM1 polymorphisms and T2DM (Hori et al.,2007 and Datta.,2010). Some studies indicated that genetic variations of the GSTT1 enzyme are associated with the development of end-stage renal disease in diabetes mellitus patients. Several studies have shown different results on the association of GST gene polymorphisms with susceptibility to T2DM in different ethnic groups.

The aim of this study is to investigate the distribution of GSTM1and GSTT1 polymorphisms in patients with type 2 diabetes mellitus in Telangana population in order to explore the possible association between GST variants and the occurrence of type 2 diabetes mellitus and also to evaluate the role of these polymorphic genes as a genetic risk modifiers in the etiology of type 2 diabetes mellitus.

MATERIALS AND METHODS

The present study included 30 patients with Type II diabetes and 30 healthy control subjects in Telangana population. The study was carried out at the Department of Genetics and Environmental Toxicology, Bhagwan Mahavir Medical Research Centre, Hyderabad, Telangana. The study was approved by the Institutional Ethics Committee of the Centre and written informed consent was obtained from all the participants of the study. After having obtained informed consent from each subject, we administered a standardized questionnaire for each participant to collect information on demographic data. 5 mL of whole blood was collected from each subject in EDTA vacutainers from the patients and control subjects, genomic DNA was isolated using salting out method (Suguna et al., 2013) and stored at -20^{o}C until use.

Multiplex PCR was performed for GSTM1 and GSTT1 polymorphisms in all the blood samples of patients and control subjects keeping globulin as an internal standard to check the gene polymorphism (Abdel et al.,1996). 25µl multiplex reaction mixture containing 25 p mol of the GSTM1 or GSTT1 primers added to 200 µmol dNTPs,5 µl of 10 x PCR buffer (10 x 500 mMKCl, 100 mMTris-HCl, pH 9.0) and 1.5 mM MgCl2 and 2 U Amplitaq gold DNA polymerase and the multiplex PCR was carried out.

The following primers were used: GSTM1 primers: (rs 4025935) FP:5'-GAACTCCCTGAAAAGCTAAAGC-3'and RP:5'-GTTGGGCTCAATATACGGTGG-3' and GSTT1 (rs 71748309) FP: 5'-TTCCTTACTGGTCCTCACATCTC-3'and RP:5'- TCACCGGATCATGGCCAGCA-3'. Human albumin gene (HAB) FP: 5'-GCCCTCTGCTAAC AAGTCCTAC-3' and RP:5'-GCCCTAAAAAGAAAATCC-CCAATC-3' primers were used as internal controls.

The PCR conditions consisted of an initial melting temperature of 94^0C for 5 min followed by 30 cycles of melting at 94^0C for 2 min, annealing at 59^0C for 1 min, extension at 72^0C for 1 min , final extension step at 72^0C for 10 min and hold at forever at 4^0c till termination. The PCR products were amplified for GSTM1, GSTT1 and globulin genes and then analyzed on agarose gel using ethidium bromide stain in 2% Agarose gel. The presence or absence of GSTM1 and GSTT1 genes was detected by the presence or absence of a band at 459 bp for GSTT1 and a band at 219bp for GSTM1. A band at 350 bp as a control of Albumin gene was every time present to show successful PCR amplification.

Statistical analysis:

Descriptive statistics have been used to describe characteristics of the study subjects by using mean, standard deviation and percentages. Chi-squire test, ORs and the 95%CIs were calculated for association studies using Graphpad prism5 and online tools.

RESULTS

The demographic data of the diabetic patients and control subjects are presented in Table-1. Out of 30 patients with diabetes 17 were (56 %) females and 13 (43.3%) were males as against 15 (50 %) females and 15 (50%) males in the control group. Mean age of diabetic patients was 31.6±5.68 yrs as against the mean age of 26.8±3.55 of control subjects. 13.3% were poor, 60% middle class and 23.3 % were rich among diabetic patients where as 43.3% were poor, 26.6% middle class and 30% were rich in the control subjects. Education qualification of patients: 33.3% high school, 26.6% graduates and 36.6% post graduates. Education qualification of control subjects: 3.33%, 63.3% and 30% respectively. 66.6% patients were married as against 33.3% among control subjects. 6.6% were vegetarians and 63.3% non-vegetarians among the patients, 93.3% were vegetarians and 36.6% were non-vegetarians in the control group. 23.3% were smokers, 76.6% nonsmokers and 16.6% were

Table-1: Demographic Details of the Subjects and Controls

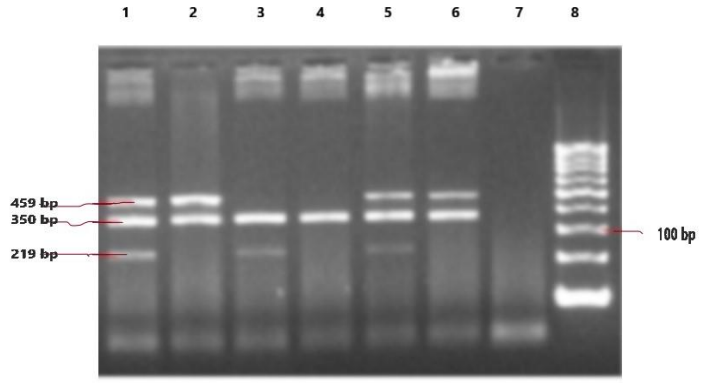
Variables	**Subjects (n = 30(%)**	**Controls (n = 30(%)**
Age (Mean±SD)	31.6±5.68	26.8±3.55
Gender		
Male	13 (43.3)	15(50)
Female	17(56.7)	15(50)
Socio Economic Status		
Poor	4(13.3)	13(43.4)
Middle	19(60.4)	8(26.6)
Rich	7(23.3)	9(30)
Education		
Below matric	0	0
High School	10(33.3)	1(3.33)
Graduation	9(30.0)	19(63.3)
Post-Graduation	11(36.7)	10(30)
Marital status		
Married	20(66.6)	16(53.3)
Un-Married	10(33.3)	14(46.6)
Food Habits		
Vegetarians	2(6.7)	19(63.3)
Non-Vegetarians	28(93.3)	11(36.6)
Smoking Status		
Smokers	7(23.3)	2(6.7)
Non-smokers	23(76.6)	28(93.3)
Alcohol Consumption (Yes/ No)		
Yes	5(16.6)	3(10)
No	25(83.3)	27(90)

alcoholics among patients as against 6.7% smokers, 93.3% non-smokers and 10% alcohol consumers among control group.

Physical characteristics of patients and control subjects are presented in Table-2. 6.6% were Physical characteristics of patients and control subjects are presented in Table-2. 6.6% were underweight, 10% with normal weight, 46.6% were overweight and 36.6% were obese in diabetic patients as against 23.3% were underweight, 66.6% normal weight, 6.6% over weight and 3.5% obese in the control subjects.

Table-2: Physical characteristics of patients and controls

Variables (BMI)	**Subjects (%) n = 30**	**Controls (%) n = 30**
Under weight (18.5)	2(6.6)	7(23.3)
Normal weight (18.5-24.99)	3(10)	20(66.6)
Over weight (25-29.99)	14(46.6)	2(6.6)
Obese	11(36.6)	1(3.3)



GSTM1 and GSTT1 polymorphisms:The GSTM1 and GSTT1 gene deletions wee analysed using Multiplex PCR.Amplicons of 219bp and 459bp indicates presence of GSTM1 and GSTT1 wild type respectively, GSTM1and GSTT1:Lane 1 and 5 represents GSTM1&GSTT1 wild type,Lane 2 & 6 :GSTT1 wild type and GSTM1 Null type, Lane 3 GSTM1 wild type and GSTT1 Null type,Lane4: GSTM1&GSTT1 null type,Lane 7: Negative control,Lane 8:100bp ladder,globin gene was used as internal control presented in 1-6 lanes.

Image –A Gel image of GSTM1 and GSTT1 gene polymorphisms of Subjects and controls

Table 3: Genotyping of GSTM1 and GSTT1 genes in Patients and Controls

Genotype	Subjects n=30 (%)	Controls n=30 (%)	OR (95%CI)	P value
GSTM1				
Wild (+)	19 (63.3)	17 (56.6)	Reference	
Null(-)	11(36.6)	13 (43.3)	0.62(0.21 to 2.02)	0.56
GSTT1				
Wild(+)	10 (33.3)	23 (76.6)	Reference	
Null(-)	20 (66.6)	7 (2.3)	0.15(0.048 to 0.50)	0.001

P= <0.05 is Significant

Table 4 : Distribution frequencies of genotype combinations between GSTM1 and GSTT1 in case and control groups and risk analysis for T2DM.

GSTT1/GSTM1	Subjects n=30 (%)	Controls n=30 (%)	OR (95%cl)	P value
(+/+)	7 (23.3)	13 (43.3)	**Reference**	
(-/+)	3 (10)	10 (30)	1.79 (0.40 to 7.52)	0.70
(+/-)	12 (40)	3 (10)	0.25(0.06 to 1.23)	0.08
(-/-)	8 (26.6)	4 (13.3)	2(0.41 to 9.42)	0.66

Multiplex Polymerase Chain Reaction was done to find the gene polymorphisms of GSTM1 and GSTT1 genes. Image-A shows gene polymorphisms of GSTM1, GSTT1 and amplicons of 219bp and 459bp and 350 bp indicated the presence of GSTM1 and GSTT1 and Globulin gene (Internal control). Genotyping of GSTM1 and GSTT1 polymorphisms in patients and controls are presented in Table -3. GSTM1 wild type was found in 63.3% of the diabetic patients and in 56.6 % of control subjects and GSTM1 null gene was found in 36.6%of the diabetic patients and in 43.3 % (P=0.56) of the control subjects while GSTT1 wild type was observed in 33.3% of the diabetic subjects and in 76.6 % control subjects and GSTT1 null type was found in 66.6% of the patients and in 23% (P=0.001) of control subjects. The statistical analysis of the results showed that GSTT1 null type is significantly associated with the risk for T2DM.The distributions of GST alleles presented in Table-4, showed the differences in the frequency of genotypes between controls and diabetes patients were statistically non-significant.

DISCUSSION

Type-2 diabetes is a multifactorial disease that develops due to environmental risk factors, (life style habits) and genetic susceptibility. It is characterized by chronic hyperglycemia and other metabolic alterations that include dyslipidemia and hypertension which lead to development of macro and micro vesicular complications. The disease pathogenesis involves a combination of beta cell in sufficiency and insulin resistance. Recent studies indicated that genetic factors play a significant role in the causation of diabetes.

GSTM1 and GSTT1 belong to super family of detoxification enzymes which protect the cells against oxidative stress and also bio transformation of xenobiotics. Oxidative stress is a potential cause of cellular dysfunction related to the various diseases including cancer, diabetes, etc., Pancreatic beta cells are more sensitive to cytotoxic stress than several other cells. Many studies reported that oxidative stress plays a pivotal role in controlling insulin production from beta cells and may be a major risk for Type 2 diabetes.

Studies were carried out from all over the world to understand the association between polymorphisms of detoxification genes and diabetes and the results are controversial.

In the present case-control study, GSTM1 and GSTT1 polymorphisms were evaluated for their association with susceptibility to T2DM and only GSTT1 null genotype was found to be associated with T2DM. Our results are in agreement with Maarib Nazih Rasheed.,(2015) who found an association of GSTT1 null genotype with diabetes in Iraqi patients. Similarly Wang, L. Zhang., (2006) reported that GSTT1 null genotype may contribute to the development of T2DM and it can be one of the candidate genes of T2DM in Chinese population. However Gonul et al., (2012) who investigated the role of GST genes as risk factor for T2DM found that GSTM1 null genotype was associated and GSTT1 null and wild genotypes were not associated with patients with T2DM in Turkish population. Yalin et al.,(2007), also reported that GSTM1 null genotype was associated with T2DM in Turkish patients. Yuille et al.,(2002) and Chen et al.,(2005) reported that both GSTM1 and GSTT1 null genotypes are risk factors for T2DM, while Adina Stoian et al.,(2015) did not find any association of GSTM1 and GSTT1 null genotypes with T2DM. Similarly Denise S. Pinheiro et al., (2013) studied influence of GSTM1 and GSTT1 deletion polymorphisms on type-2 diabetes mellitus risk in Brazilian population and suggested that the null type of GSTM1 and GSTT1 genes may cause T2DM.

These findings also demonstrate the complex nature of T2DM. This is recognized as a disease that involves alterations in various clinical parameters, which need to be constantly monitored in order to avoid the complications.

CONCLUSION

The preliminary results suggest that GSTT1 null genotype may contribute to the development of T2DM in Telangana population. However further studies in a larger sample size are warranted to arrive at definitive conclusion.

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