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ASSOCIATION OF XENOBIOTIC GENETIC VARIANTS WITH OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT
Xenobiotics are defined as any foreign substances or exogenous chemicals which the body does not recognize such as drugs, pollutants, as well as some food additives and cosmetics. The body removes xenobiotics by xenobiotic metabolism. This consists of the deactivation and the excretion of xenobiotic and happens mostly in the liver. Hepatic Microsomal Cytochrome P450 is a group of enzymes involve in xenobiotic metabolism. The increased oxidative stress is one of the main factors in the etiology and complications of Diabetes Mellitus. Three of the GST (glutathione-S-transferase) genes, GSTM1, GSTT1 and GSTP1 have been found to have functional polymorphisms that are frequently present in the general population. GSTM1 and GSTT1 polymorphisms are the most common polymorphisms of GST enzymes in the human population with major ethnic differences. In our study, among two xenobiotic genetic variants GSTT1 and GSTM1, no association of GSTT1 was found, but the variant GSTM1 was found to be risk in Type 2 Diabetes Mellitus.

KEYWORDS: Xenobiotics, GST genes, Type 2 Diabetes Mellitus.

INTRODUCTION
Xenobiotics are defined as foreign substances or exogenous chemicals which the body does not recognize, such as drugs, pollutants, as well as some food additives and cosmetics. The body removes xenobiotics by xenobiotic metabolism. Xenobiotic metabolism is the principle pathway that scavenges lipophilic xenobiotic agents and certain endogenous metabolites using phase I and phase II enzymes. Any alteration in this metabolic pathway prevents scavenging and increase reactive intermediates that have the potential of oxidative damage. The increased oxidative stress is one of the main factors in the etiology and complications of Diabetes Mellitus (DM).[1] Glutathione (GSH) is the major cellular antioxidant that protects against environmental toxicants as well as Reactive Oxygen Species (ROS) mediated cell injury. GSH detoxifies ROS, reduces peroxides and detoxifies multiple compounds through glutathione-S-transferase (GST) conjunction.[2] GSTs belong to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemicals, metabolites and oxidative stress.[1] An important condition affecting GST expression is oxidative stress, usually observed in DM.[3] Three of the GST genes, GSTM1, GSTT1 and GSTP1 have been found to have functional polymorphisms that are frequently present in the general population. GSTM1 and GSTT1 polymorphisms (xenobiotic genetic variants) are the most common polymorphisms of GST enzymes in the human population with major ethnic differences and have been studied most extensively in many studies. Five μ (mu) class genes are situated (GSTM1 - GSTM5) on chromosome 1.[4] There are two θ (theta) class genes, GSTT1 and GSTT2, located on chromosome 22.[5] In view of limited data available this study was conducted to investigate the distribution of GSTM1 and GSTT1 polymorphisms in Type 2 DM patients to explore the possible association between GST variants and oxidative stress.

MATERIALS AND METHODS
The blood samples of 42 T2DM cases and 34 controls were randomly taken for the study of xenobiotic genetic variants with the age group between 31 to 60 years. The extraction of DNA was done by salting out method. The quantitative and qualitative evaluations of genomic DNA were done by spectrophotometric method and gel electrophoresis method.

Analysis of single nucleotide polymorphisms
GST polymorphism
Deletion status of GSTM1 and GSTT1 was simultaneously determined by a multiplex polymerase chain reaction method. GSTM1 and GSTT1genes were amplified using the following primers: 5′GAA CTC CCT GAA AAG CTA AAG C 3′ (forward) and 5′GTT GGG CTC AAA TAT ACG GTG G 3′ (reverse) for GSTM1

and 5′TTC CTT ACT GGT CCT CAC ATC TC 3′ (forward) and 5′TCA CCG GAT CAT GGC CAG CA 3′ (reverse) for GSTT1. As an internal control MTHFR C677T gene was co-amplified using the primers 5'-TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG-3 (forward) and 5'-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3' (reverse). Each 25 µL of PCR mixture was composed of 14.2 µL double distilled water, 2.5 µL 10 X buffer B, 3.0 µL 50 mm $MgCl_2$, 1.0 µL 10 mm dNTP, 0.1 µL of each primer for GSTT1, GSTM1 and MTHFR C677T respectively, 1.2 µL DMSO, 0.5 µL Taq and 2 µL genomic DNA. The PCR conditions were: initial denaturation at 94^o C for 5 min followed by 35 cycles of denaturation at 94^o C for 1 min, annealing at 64^o C for 1 min, extension at 72^o C for 1 min and a final extension of 72^o C for 7 min. Agarose gel electrophoresis (1%) resolved amplified DNA fragments of 480, 173 and 215 bp for GSTT1, MTHFR C677T and GSTM1 respectively.

Quality control

All the PCR reactions were accompanied by a negative control without genomic DNA to rule out the possibility of genomic DNA contamination of reagents. All the RFLP analysis was accompanied by negative and positive controls that lack or have restriction site of the target restriction enzyme. Approximately 10% of samples were reanalyzed to rule out genotyping errors and found 100% concordance.

RESULTS

Genotypes and allele frequency distribution of xenobiotic genetic variants in T2DM cases and controls ate shown in Table 1. The allele frequency distribution of xenobiotic genetic variants in T2DM cases and healthy controls were as follows: GSTT1 (33% vs 26%) and GSTM1 (19% vs 12%). PCR Conditions for the analysis of xenobiotic genetic polymorphisms are shown in Table 2. Genotypes and allele frequency distribution of xenobiotic genetic variants in T2DM cases and controls are shown in Table 3. Among two xenobiotic genetic variants GST T1 and GST M1, the variant GST M1 was found to be risk in T2DM (OR: 39.91; 95 % CI: 4.14-384.88). Correlation of xenobiotic genetic variants with oxidative stress markers is shown in Table 4. Among control population GST T1 showed positive correlation with NO (r=0.31; p=0.05) and GSH (r=0.50; p=0.008). As per Table 5, the Logistic regression analysis of xenobiotic genetic variants shows that among two xenobiotic genetic variants, only GSTM1 was found to be risk for endothelial dysfunction in T2DM.

Table 1: Genotypes and allele frequency distribution of xenobiotic genetic variants in T2DM cases and controls.

SNPs	Genotypes		Minor allele frequency	Adjusted OR (95 % CI)	p Value
GSTT1	**0**	**1**			
Cases	14	28	14/42 (33%)	1.03(0.91-1.16)	0.68
Controls	9	25	9/34 (26%)		
GSTM1					
Cases	8	34	8/42 (19%)	39.91(4.14-384.88)	0.001*
Controls	4	30	4/34 (12%)		

Adjusted OR: adjusted odds ratio for age; CI: confidence interval; 0: presence of allele; 1: null genotype; *: statistically significant

Table 2: PCR Conditions for the analysis of xenobiotic genetic polymorphisms.

Polymorphism	Primers (5'-3')	PCR Conditions	Method
GSTT1	TTC CTT ACT GGT CCT CAC ATC TC TCA CCG GAT CAT GGC CAG CA	D: 94^o C 1min A: 64^o C 1min E: 72^o C 1min 35 X	Multiplex PCR
GSTM1	GAA CTC CCT GAA AAG CTA AAG C GTT GGG CTC AAA TAT ACG GTG G		

D: denaturation; A: annealing; E: extension; X: number of cycles

Table 3: Genotypes and allele frequency distribution of xenobiotic genetic variants in T2DM cases and controls

SNPs	Genotypes		Minor allele frequency	Adjusted OR (95 % CI)	p Value
GSTT1	**0**	**1**			
Cases	14	28	14/42 (33%)	1.03(0.91-1.16)	0.68
Controls	9	25	9/34 (26%)		
GSTM1					
Cases	8	34	8/42 (19%)	39.91(4.14-384.88)	0.001*
Controls	4	30	4/34 (12%)		

Adjusted OR: adjusted odds ratio for age; CI: confidence interval; 0: presence of allele; 1: null genotype; *: statistically significant

Table 4: Correlation of xenobiotic genetic variants with oxidative stress markers

Variants	NO		MDA		GSH	
	r	p	r	p	r	p
GST T1						
Cases	-0.07	0.71	0.10	0.56	-0.02	0.89
Controls	0.38	0.05*	-0.53	0.005*	0.50	0.008*
GST M1						
Cases	-0.16	0.36	0.24	0.17	-0.09	0.62
Controls	-0.01	0.96	-0.11	0.57	0.16	0.42

r=Pearson's correlation coefficient; * P < 0.05: statistically significant

Table 5: Logistic regression analysis of xenobiotic genetic variants

Variants	Coefficient	Std Err	OR (95% CI)	p value
GSTT1	0.02	0.06	1.03(0.91-1.16)	0.68
GSTM1	3.68	1.15	39.91(4.14-384.88)	0.001*

OR: odds ratio; CI: confidence interval; * P < 0.05 statistically significant

DISCUSSION

Hyperhomocysteinemia generate ROS through multiple mechanisms: (1) inhibition of the activity of cellular antioxidant enzymes such as cellular glutathione peroxidase or heme oxygenase -1, (2) HCY autooxidation, (3) NOS-dependent generation of superoxide anion ($O_2^{-\cdot}$) via uncoupling of eNOS, (4) decrease of extracellular superoxide dismutase activity and (5) by phosphorylation at p47 phox and p67 phox subunits of NADPH oxidase, thereby stimulating superoxide generation.[6]

One-carbon metabolism plays a crucial role in Phase II of xenobiotic metabolism by supplying (i) methyl group for O-methylation of catechol derivatives and (ii) GSH for GST-mediated conjugation step. Any perturbation in this pathway might influence Phase II reactions. GSTs can work as endogenous antioxidants to protect cells from oxidative stress. The GSTs catalyze the conjugation of GSH to a wide range of electrophiles and represent a protective mechanism against oxidative stress. The GST family of genes is critical in the protection of cells from ROS because they utilize as substrates a wide variety of products of oxidative stress. Studies have shown an independent association of GSTT1 and GSTM1 null variants with CAD.[7] The various early studies were: the risk associated with GSTT1 and GSTM1 null variants to dyslipidemia[8]; the independent association of GST T1 null variant with CAD and synergetic effect with GST M1 null on disease severity[9]; association of the combined genotype of GSTT1 null/GSTM1 null with disease severity in smokers[10]; GSTT1 and GSTM1 null genotypes is independent of smoking[11]; increased DNA damage in smokers carrying GSTT1 and GSTM1 null alleles compared to smokers having wild genotype at these loci.[12] Altered GST activity due to genetic mutations has been shown to modulate individual's susceptibility to environmental factor induced diseases; including cancers.[13] GSTT1-null genotype (homozygous for non-functional allele) has a decreased capability in detoxifying some carcinogens and oxygen metabolites. It is possible, therefore, that the effect of the GSTT1 genotype is the same in the development of cancer and DM. Therefore, it might be indicated that the GSTT1 present genotype is protective against T2DM. The genetic contribution of GSTs to diabetes has been previously investigated; GST polymorphisms have been reported to be associated with trogitazone response in T2DM.[14] Another study reported the lack of direct association between GSTT1 and T2DM.[15] In our study, among two xenobiotic genetic variants GSTT1 and GSTM1, no association of GSTT1 was found, but the variant GSTM1 was found to be risk in T2DM.

CONCLUSION

Among control population GSTT1 showed positive correlation with NO and GSH. The Logistic regression analysis of xenobiotic genetic variants shows that among two xenobiotic genetic variants, only GSTM1 was found to be risk for endothelial dysfunction in T2DM.

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