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Evaluation of glutathione S-transferase T1 deletion polymorphism on type 2 diabetes mellitus risk in Zoroastrian females in Yazd, Iran

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Abstract

Background:

There has been much interest in the role of free radicals and oxidative stress in the pathogenesis of diabetes mellitus (DM). The aim of this study was to assess the possible association between genetic polymorphisms of the glutathione S-transferase-Theta (GSTT1) and the risk of the development of DM in Zoroastrian females in Yazd, Iran.

Materials and Methods:

This was a case-control study in which GSTT1 polymorphism was genotyped in 51 randomly selected DM patients and 50 randomly selected healthy controls among Zoroastrian females whose ages ranged from 40 to 70.

Results:

The frequencies of GSTT1 null genotype and GSTT1 present were 72% and 28%, respectively, in control samples, while in patients with type 2 diabetes (T2DM), the frequencies of GSTT1 null genotype and GSTT1 present were 27.5% and 72.5%, respectively. There were higher levels of triglyceride (TG), fasting blood sugar (FBS), total cholesterol (TC), low-density lipoprotein (LDL), Urea, and high-density lipoprotein (HDL) in cases of GSTT1 null genotype compared to the GSTT1 present genotype in controls.

Conclusions:

Our results indicated that healthy subjects had a higher frequency of the GSTT1 null genotype than patients with T2DM. However, we observed no significant association between the GSTT1 null genotype and T2DM in the current study.

Keywords: Ethnic group, female, glutathione S-transferase T1, genetic polymorphism, type 2 diabetes, Zoroastrian

INTRODUCTION

Diabetes mellitus (DM), which is estimated that 347 million adults are suffering from,[1] has become an important cause of mortality and morbidity worldwide, through both direct clinical sequelae and increased

mortality from cardiovascular and kidney diseases.[2,3,4,5] DM results from body's ineffective use of insulin, which is determined by several different genes and environmental factors. Causes of the DM are both various and complex, and one of these causes is oxidative stress, arising as a result of an imbalance between free radicals and antioxidant defenses.[6] As β-cells are very sensitive to cytotoxic stress because of their little expression of the antioxidant enzymes, they are susceptible to the oxidative stress attack, and the dysfunction of β-cells after oxidative stress attack may further result in the development of DM.[7] Glutathione S-transferases (GSTs) are the most important family of phase II isoenzymes known to detoxify a variety of electrophilic compounds, including carcinogens, chemotherapeutic drugs, environmental toxins, and DNA products generated by reactive oxygen species damage to intracellular molecules, chiefly by conjugating them with glutathione.[8] GSTs play a major role in cellular antimutagen and antioxidant defense mechanisms.[9] Glutathione S-transferase T1 (GSTT1) gene is polymorphic in human and the null genotype result in the absence of enzyme function, contributing to interpersonal differences in response to xenobiotics. In recent years many studies have assessed the associations between DM and GSTT1 polymorphism.[10,11,12,13,14,15] Ramprasath *et al*. demonstrated significant associations between GSTM1/GSTT1 null genotypes and DM risk,[14] and similar results were also reported in other studies.[10] However, some studies reported different conclusions and showed that there were no obvious associations between GSTT1 null genotype and DM risk,[11,13] or GSTT1 caught the associations.[12,15] Thus, it remains unclear whether there are significant associations between GSTT1 polymorphism and DM risk. Although Iranian people are mostly Muslims, an ethno-religious minority of people who practice Zoroastrianism live in Iran, representing approximately 0.02-0.05% of the population. Zoroastrianism originated between the ninth and sixth centuries BC, and it was introduced by Sassanid as the official religion during the last pre-Islamic Persian Empire.[16] In the last millennium, Zoroastrians have lived in a high level of isolation as well as endogamy, and this condition has been maintained vigorously to date. This enabled the survival of most of the mtDNA of their indigenous Iranian ancestors due to the lack of foreign contributions to their gene pool in the recent past,[17] thus providing an outstanding opportunity to study risk factors associated with type 2 diabetes (T2DM) in a limited genetic-variability setting.

The aim of this study was to evaluate whether GSTT1 polymorphism is associated with T2DM among the Zoroastrian female population. The rationale for the study was that the contribution of the GSTT1 polymorphism to the risk of the development of T2DM is currently unknown. To the best of our knowledge, no study has yet investigated the role of GSTT1 polymorphism and T2DM risk in the Zoroastrian population in Yazd, Iran.

Materials and Method

Study population

In this case-control study conducted at the Yazd Diabetes Research Center, 51 women with T2DM were selected from the subjects who participated in a community-based, cross-sectional study of the Zoroastrians living in Yazd, Iran. In that cross-sectional study, 51 women met the criteria established by the American Diabetes Association (ADA) for a diagnosis of diabetes, and all of them enrolled in the current study. To facilitate equal sampling, a control group of 50 healthy women who did not meet ADA's criteria for a diagnosis of diabetes were selected randomly from the same geographic region. The size of the sample was determined by the following formula for a one-sided hypothesis test:

$$n = \frac{(Z_{(1-\alpha)}\sqrt{2\bar{P}(1-\bar{P})} + Z_{(1-\beta)}\sqrt{P_1(1-P_1)+P_2(1-P_2)})^2}{(P_1-P_2)^2}$$

$$= \frac{(0.668+0.7191)^2}{(0.18)^2} = 45$$

$$\bar{P} = \frac{P_1+P_2}{2}$$

Where n is the size of the sample, Z = 1.64, is the significance level of the test (0.05), is the probability of failing to detect a shift of one standard deviation (0.20), P1 is the proportion for control group (0.12), and

P2 is the proportion for the cases (0.29). The values of P1 and P2 were hypothesized based on past studies.

Anthropometric variables and biochemical assays of blood

The subjects' weights were measured to the nearest 0.1 kg using a calibrated scale (Seca 220, Seca GmbH & Co. KG., Hamburg, Germany) with the subjects wearing light clothing and standing in an upright position. The subjects' heights were measured to the nearest 0.5 cm using a standard stadiometer (Seca 220, Seca GmbH & Co. KG., Hamburg, Germany) while the subjects were not wearing shoes. BMI was calculated by dividing weight (kg) by height squared (m2). After a 10-minute rest, the subjects' blood pressure (BP) was measured twice (on a single occasion) by a standard mercury sphygmomanometer. The measurements were made to an accuracy of the nearest 2 mmHg while the subjects were in a seated position. After 12-14 hours of overnight fasting, venous blood samples were taken from the subjects and analyzed in the laboratory of the Yazd Diabetes Research Center. An oral glucose tolerance test (OGTT) was conducted using 75-gm oral glucose powder. Blood levels of glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), urea, creatinine (Cr), and uric acid were measured by an autoanalyzer (AMS Autolab, Italy) using pertinent Pars Azmun kits (Pars Azmun Co, Tehran, Iran), that is, GOD-PAP for glucose, CHOD-PAP for TC, GPO-PAP for TG, ENZYMATIC for LDL, and PERCIPITANT for HDL. The latest criteria established by the ADA were used for the diagnosis of DM in the subjects.[18]

DNA extraction and genotyping

Blood was collected into EDTA-containing tubes, and DNA was extracted from the lymphocytes using a high-purity template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany). The characterization of GSTT1 polymorphism was performed using a real-time polymerase chain reaction (PCR) with a Light Cycler instrument and hybridization probes in combination with the Light Cycler DNA master hybridization probes kit (Roche Diagnostics). Both the PCR primers and hybridization probes were synthesized by TIB MOLBIOL (Berlin, Germany). The PCR conditions were included 4 mmol/l of MgCl2 (magnesium chloride), 0.2 mmol/l of each hybridization probe, 10 pmol of each PCR primer, 2 μl of the Light Cycler DNA master hybridization mix, and 50 ng of genomic DNA in a final volume of 20 μl. The fluorescence signal was plotted against temperature to give melting curves for each sample.

The study's protocol was approved by the Medical Ethics Committee of Yazd Islamic Azad University of Medical Sciences. Written informed consent forms were collected from all participants.

Statistical analyses

Allele distributions were compared using chi-squared tests. The student's *t*-test was used to determine differences in the means of age. $P < 0.05$ were considered statistically significant. The associations of the GSTT1 polymorphism in study groups and control subjects were modeled using binary logistic regression analysis. Odds ratios (ORs) and confidence intervals (CIs) were used to analyze the relationship of the GSTT1 genotype in patients with T2DM compared to the control groups. SPSS version 17 (SPSS Inc., Chicago, IL, USA) was used for the analyses of the data.

RESULT

A total of 101 individuals (51 patients with DM and 50 controls) were genotyped for the GSTT1. The frequency distribution of GSTT1 genotype in healthy subjects and patients was determined by using real-time PCR. The mean ages of the patients and controls were 61.7±9.4 and 52.7±11.2, respectively.

In the control samples, the frequency of GSTT1 null genotype and GSTT1 present was 72 and 28%, respectively, while in patients with T2DM, the frequency of GSTT1 null genotype and GSTT1 present was 27.5 and 72.5%, respectively (OR = 1.01, 95% CI = 0.41-2.40, $P = 0.98$) [Table 1].

Anthropometric and metabolic variables according to GSTT1 genotype

We further investigated the clinical parameters accompanying high risk genotype (GSTT1 null genotype) compared to non-risk genotype (GSTT1 present genotype) in patients and controls [Table 2]. In cases of

GSTT1 null genotype, there were higher levels of TG, fasting blood sugar (FBS), TC, LDL, Urea, Cr and HDL compared to the GSTT1 present genotype in controls. In patients, there were higher levels of TC, HDL, and LDL in GSTT1 present genotype compared to the GSTT1 null genotype. We also showed that in patients with GSTT1 null genotype, there were higher levels of TG, FBS, Urea, Cr and BMI compared to the GSTT1 null genotype in controls.

Discussion

In this case-controlled study, GSTT1 deletion polymorphism was evaluated for its association with susceptibility to T2DM. The distributions of the GSTT1 null genotypes were not significantly different for the patients and the control group. The deletion frequency of GSTT1 in the control group (72%) was greater than the frequencies obtained in a study conducted by Arruda *et al*. in Brazil, that is, 18% to 20%, which might have been due to ethnic differences among regions of the Brazilian population.[19] In addition, patients with diabetes had a higher frequency of the GSTT1 null genotype (29.2%) than healthy subjects (12.2%). Our study showed that the GSTT1 null genotype resulted in 1.01-fold increased risk for T2DM. Thus, individuals may have decreased antioxidant defenses when this isoform was deleted. Furthermore, it has been well documented that a GSTT1 present genotype can confer protection against the development of T2DM.[10,20,21] These results suggest that the GSTT1 deletion polymorphism may play a role in the pathogenesis of T2DM. It was also found that there was no association of GSTM1 with susceptibility to T2DM. There are studies that reported significant association to T2DM for both null genotypes of GST[10,14] and others that verified no association between GSTT1 and GSTM1 polymorphisms and T2DM.[13,21] In addition, others studies showed that only the GSTM1 null genotype may play a significant role in the etiopathogenesis of T2DM.[12,15] In the Turkish population study,[15] the authors suggested that the GSTM1 genotype may be a useful marker in the prediction of T2DM susceptibility. The OR obtained for the GSTM1 null genotype was 3.7, indicating an association between the incidence of diabetes and GSTM1 deletion polymorphism. In accordance, an Indian population study reported a significant association of GSTM1 null (OR = 2.042) with T2DM and no significant association with GSTT1.[12] Despite some divergence in the literature data, GSTT1 null and GSTT1 null/GSTM1 null genotypes have consistently been considered risk factors for the development of T2DM as reported by a meta-analysis study.[22] In a study conducted by Amer *et al*.,[10] the authors found significant differences between the double present genotype (+ / +) and either or both null genotypes of diabetics ($P = 0.002$ and $P = 0.009$, respectively) when compared to the control subjects. They confirm that GSTT1 and GSTM1 cooperatively play a protective role against the development of T2DM. Furthermore, in the Indian study,[14] the results implied that there was a 1.84 increased risk for T2DM with the combination of either null genotypes of GSTM1/GSTT1 (+ /2 or 2/ +). The evaluation of clinical variables association with GST polymorphism in diabetic patients showed that the GSTT1 null genotype relates to significantly higher levels of TG and HDL when compared to the present genotype. This allows us to infer that the absence of GSTT1 may contribute to type 2 diabetes-related complications, such as dyslipidemia. These results are consistent with studies conducted on the Chinese population,[20] Egyptian population[10] and Indian population,[14] where a GSTT1 null association with lipid alterations was also observed. Thus, the GSTT1 gene could be added to a set of potential genetic markers to identify individuals at increased risk for developing T2DM and complications associated with dyslipidemia in diabetic patients. While a significant relationship between GSTT1 deletion polymorphism and susceptibility to disease was not verified, it was possible to observe the influence of this polymorphism on clinical parameters related to TG and HDL. Therefore, the deletion of GSTT1 genotype can have relevance in the clinical course of diabetic patients, since those two variables, along with lipid profile, are focal points for disease monitoring to prevent T2DM complications. The mechanisms underlying the results of association obtained in this and other works still need to be investigated with further research.

This study has various limitations. First, the small number of subjects was a major limitation. Therefore, the study may not have had enough power to clarify whether the GSTT1 polymorphism is related with risk of acquiring DM, and future studies with larger patient samples with different genders and a longitudinal design are necessary. These findings may not be generalizable to other populations, given that differences in racial and ethnic attitudes toward lifestyle may influence these results. As a strength, to the best of our

knowledge, this is the first study that has investigated association between GSTT1 polymorphism and DM in the Zoroastrians (a minority group) who live in Yazd, Iran.

The most obvious finding to emerge from this study was that healthy subjects had a higher frequency of the GSTT1 null genotype than diabetic patients. However, we observed no significant association between the GSTT1 deletion polymorphism and T2DM in the current study. It is recommended that future studies investigate the role of the GSTT1 and its combination with other GSTs genotypes in the pathogenesis of DM and its associated complications in large-scale cohorts in different populations.

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Footnotes

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Figures and Tables

Table 1

Locus	Genotype	Patient (*n*=51) (%)	Control (*n*=50) (%)	Odds ratio (95% CI)	*P*
GSTT1	Present	37 (72.5)	14 (28)	1.00 (ref)	
	Null	14 (27.5)	38 (72)	1.01 (0.41-2.40)	0.98

n: Number of sample, DM: Diabetes mellitus

Association between GSTT1 genotype profile and the development of DM

Table 2

Clinical parameters	Case			Control		
	Present (*n*=25)	Null (*n*=26)	*P* value	Present (*n*=36)	Null (*n*=14)	*P* value
Age (years)	61.8±9.9	61.4±8.6	0.892	51.5±10.6	56±12.5	0.209
FBS (mg/dl)	120.9±23.4	135±47.7	0.166	98.2±16.4	99.5±17	0.816
TG (mg/dl)	163.9±77.9	192±56.6	0.226	145.2±53	183.6±43.7	0.02
TC (mg/dl)	200.4±35	192.2±34	0.457	203±40.3	216.2±40.5	0.302
LDL (mg/dl)	126.1±22	109.7±26.9	0.031	130.4±22.3	136.5±25.2	0.409
HDL (mg/dl)	39.3±10.4	35.9±6	0.261	38.9±7.4	44±8.9	0.049
Urea (mg/dl)	33.2±7.1	37.4±14.7	0.176	31.5±6.9	32.8±7.4	0.547
Cr (mg/dl)	0.90±0.17	0.96±0.29	0.355	0.92±0.16	0.95±0.13	0.562
BMI (kg/m^2)	26.5±3.9	26.8±4.8	0.885	25.8±3.2	23.9±4.2	0.091

Data are reported as means±S.D. *n*: number of sample, FBS: Fasting blood glucose, TG: Triglyceride, TC: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, Cr: Creatinine, BMI: Body mass index

Anthropometric and metabolic variables according to GSTT1 genotype