Evaluation of Glutathione S-transferase T1 (GSTT1) deletion polymorphism on type 2 diabetes mellitus risk in a sample of Yazdian 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-mu (GSTT1) and the risk of the development of DM in a sample of Yazdian females in Yazd, Iran.
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 Yazdian females whose ages ranged from 40 to 70.
Results: The frequencies of GSTT1 null genotype and GSTT1 present were 8 and 92%, respectively, in the control samples. In patients with type 2 diabetes (T2DM), the frequencies of GSTT1 null genotype and GSTT1 present were 14 and 86%, respectively. There were higher levels of triglycerides (TG), fasting blood sugar (FBS), total cholesterol (TC), low density lipoprotein (LDL), body mass index (BMI), and high density lipoprotein (HDL) in patients with GSTT1 null genotype than in patients with the GSTT1 present genotype.
Conclusions: Our results indicated that the GSTT1 deletion polymorphism is a risk factor for T2DM. We did not determine any significant association between the GSTT1 null genotype and T2DM.

Keywords: glutathione S-transferase T1, genetic polymorphism, type 2 diabetes, female, Iran

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1. Introduction

1.1. Background

It is estimated that 347 million adults in the world suffer from diabetes mellitus (DM) (1); DM has become an important cause of mortality and morbidity worldwide resulting from its direct clinical sequelae and increased mortality due to associated cardiovascular and kidney diseases (2-5). DM results from the body's ineffective use of insulin, which is determined by several different genes and environmental factors. The causes of DM are both various and complex, and one of these causes is oxidative stress, which arises as a result of an imbalance between

free radicals and antioxidant defenses (6). Since β-cells are very sensitive to cytotoxic stress because of their little expression of the antioxidant enzymes, they are susceptible to the oxidative stress, and the dysfunction of β-cells after undergoing oxidative stress may 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 the damage caused to intracellular molecules by reactive oxygen species, chiefly by conjugating them with glutathione (8). GSTs play a major role in cellular antimutagen and antioxidant defense mechanisms (9). The glutathione S-transferase T1 (GSTT1) gene is polymorphic in humans, and the null genotype result in the absence of enzyme function contributes to the interpersonal differences in response to xenobiotics.

1.2. Statement of the problem and objectives

The prevalence and incidence of type 2 diabetes mellitus (T2DM) are high in the Middle Eastern countries (10), and it has been estimated that these countries will have the largest increases in the prevalence of diabetes by 2030 (11).The prevalence of T2DM was determined to be 14.52% in Yazd, Iran (12). Environmental factors, such as urbanization and subsequent westernization of lifestyles, and genetic susceptibility are considered as possible etiologies for the T2DM epidemic in Asia (13). In recent years, many studies have assessed the associations between DM and GSTT1 polymorphism (14-19). Ramprasath and colleagues demonstrated significant associations between GSTM1/GSTT1 null genotypes and DM risk (18), and similar results also were reported in other studies (14). However, some studies reported different conclusions and showed that there were no obvious associations between GSTT1 null genotype and DM risk (15-19). In Yazd, Dadbinpour examined the deletion of GSTT1 and GSTM1 genes in 57 diabetic patients with retinopathy and 58 diabetic patients without retinopathy. They indicated that there was a significant relationship between GSTM1 null genotype with the retinopathy side effect of T2DM. However, there was no significant relationship between GSTT1 null genotypes with retinopathy in T2DM (20). Thus, it remains unclear whether there are significant associations between GSTT1 polymorphism and the risk of DM. Recently, the GSTT1 null genotype interacting with current-smoking status was shown in two different studies to be a genetic risk factor for the development of T2DM and its cardiovascular complications (17, 18). In the Sinai area of Egypt, in a study of 100 T2DM patients and 100 healthy controls, matched for age, gender, and origin, the proportion of the GSTT1 null genotype was significantly greater in the diabetic patients than in the controls. It was reported that the risk of having T2DM increased by a factor of 3.17 in patients who had null polymorphism compared to those with the normal genotype of this gene (P = 0.009) (19). The rationale for the study was that the contribution of GSTT1 polymorphism to the risk of the development of T2DM is currently unknown. The aim of this study was to determine the association between the GSTT1 genotype and T2DM among the sample of the female population of Yazd, Iran.

2. Material and Methods

2.1. Study setting and design

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 Yazdian population of 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, α is the significance level of the test (0.05), β is the probability of failing to detect a shift of one standard deviation (0.20), p_1 is the proportion for control group (0.12), and $p_2$is the proportion for the cases (0.29). The values of p_1 and p_2 were hypothesized based on past studies.

2.2. 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 the subjects' weights (kg) by their height squared (m^2). After a 10-minute rest, the subjects' blood pressures (BPs) were measured twice (on a single occasion) by a standard mercury sphygmomanometer. The measurements were made to an accuracy of the nearest 2 mmHg with the subjects 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 a 75-gm oral dosage of glucose powder. Blood levels of glucose, triglycerides (TGs), 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), i.e., 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 (21).

2.3. 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 essentially the same as those described by Ko and colleagues (22) and included 4 mmol L^{-1} of $MgCl_2$ (magnesium chloride), 0.2 $mmolL^{-1}$ 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.

2.4. Ethical considerations

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

2.5 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 values < 0.05 were considered statistically significant. The associations of the GSTT1 polymorphism in the study group and in the 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, Illinois, United States) was used to analyze the data.

3. Results

A total of 101 individuals (51 patients with T2DM 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 52.4±11.2 and 59.4±9.9, respectively.

3.1. Association between GSTT1 genotype profile and the development of T2DM

In the control samples, the frequencies of GSTT1 null genotype and GSTT1 present were 8 and 92%, respectively, while in the patients with T2DM, the frequencies were 14 and 86%, respectively (OR = 1.97, 95% CI = 0.51-7.52, P = 0.31) (Table 1).

3.2. Anthropometric and clinical variables according to GSTT1 genotype

We investigated the clinical parameters that accompanied the high risk genotype (GSTT1 null genotype) compared to non-risk genotype (GSTT1 present) in patients and controls (Table 2). The participants who had the GSTT1 present genotype had higher levels of fasting blood sugar (FBS), TC, Urea, Cr, BMI, and HDL than the GSTT1 null genotype in the controls. In the patients, there were higher levels of TC, TG, FBS, HDL, and LDL in the GSTT1

null genotype than in the GSTT1 present genotype. We also showed that there were higher levels of TG, LDL, FBS, Urea, Cr, and BMI in the controls with GSTT1 null genotype than in the GSTT1 null genotype in the patients.

Table 1. Association between GST genotype profile and the development of metabolic syndrome

Locus	genotype	Patients (n = 51) (%)	Controls (n = 50) (%)	Odds ratio (95% CI)	P-value
GSTT1	Present	43 (86)	46 (92)	1.00 (Ref)	
	Null	7 (14)	4 (8)	1.97 (0.51-7.52)	0.31

n: number of sample

Table 2. Anthropometric and metabolic variables according to GSTM1 genotype

Clinical parameters	Case			control		
	Present (n = 43)	Null (n = 7)	P-value	Present (n = 46)	Null (n = 4)	P-value
Age (years)	52±11.1	55±12.5	0.519	51.5±10.6	58.7±6.8	0.884
FBS (mg/dl)	98.1±15.8	101.5±20.7	0.613	103.3±9	103.2±13.8	0.816
TG (mg/dl)	154±52.8	167.5±57.3	0.539	185.7±43.8	201.2±58.8	0.511
TC (mg/dl)	204.7±38.9	219±50	0.392	123.6±22.6	108.2±36.7	0.220
LDL (mg/dl)	131.2±22.1	137.2±29.7	0.529	171.2±76	187±42.7	0.686
HDL (mg/dl)	40±7.8	42.7±9.8	0.419	38.7±9.8	34.7±5.9	0.433
Urea (mg/dl)	32.1±7.01	30.4±7.7	0.176	33.4±6.3	30.2±4	0.197
Cr (mg/dl)	0.93±0.16	0.88±0.06	0.40	0.98±0.2	0.90±0.02	0.689
BMI (kg/m^2)	25.7±3.1	22.5±5.1	0.026	26.7±4.2	25.3±3.6	0.527

Data are reported as means ± S.D.; n: number of samples; FBS: fasting blood glucose; TG: triglyceride; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; Cr: creatinine; BMI: body mass index

4. Discussion

4.1. Association between GSTT1 genotype profile and the development of T2DM

In this case-controlled study, GSTT1 deletion polymorphism was evaluated for its association with susceptibility to T2DM. We demonstrated an association of the GSTT1 null genotype with an increased risk of 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 (8%) was lower than the frequencies obtained in a study conducted by Arruda and colleagues in Brazil, i.e., 18 to 20%, which might have been due to ethnic differences among regions of the Brazilian population (23). In addition, diabetic patients 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 almost a two-fold increase in the risk T2DM. Thus, individuals may have decreased antioxidant defenses when this genotype was deleted. Furthermore, it has been well documented that a GSTT1 present genotype can provide protection against the development of T2DM (14, 24, and 25). These results suggest that the GSTT1 deletion polymorphism may play a role in the pathogenesis of T2DM. It also was found that there was no association of GSTT1 with susceptibility to T2DM. There are studies that have reported significant association between both null genotypes of GST and T2DM (14, 18), but others have indicated that there is no association between GSTT1 and GSTM1 polymorphisms and T2DM (17, 25). In addition, others studies have shown that only the GSTM1 null genotype may play a significant role in the aetiopathogenesis of T2DM (16, 19). In a study of the Turkish population (19), the authors suggested that the GSTM1 gene 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 the GSTM1 deletion polymorphism, and a study of the population in India reported a significant association of the GSTM1 null genotype (OR = 2.042) with T2DM and no significant association with GSTT1 (16).

Despite some divergence in the data in the literature, the GSTT1 null genotype and the GSTT1 null/GSTM1 null genotypes consistently have been considered risk factors for the development of T2DM, as reported by a meta-analysis study (26). In a study conducted by Amer and colleagues (14), 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 confirmed that GSTT1 and GSTM1 cooperatively play a protective role against the development of T2DM. Furthermore, in the Indian study (18), the results implied that the risk for T2DM was almost doubled with the combination of either null genotypes of GSTM1/GSTT1 (+ /2 or 2/ +).

4.2. Anthropometric and clinical variables according to GSTT1 genotype

The evaluation of the association of clinical variables with GST polymorphism in diabetic patients showed that the GSTT1 null genotype relates to significantly higher levels of FBS, TC, TG, LDL, and HDL than 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 (24), the Egyptian population (14), and the Indian population (18), in which a GSTT1 null genotype association with lipid alterations also was 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 the 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 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 studies still should be investigated in future research.

4.3. Study limitations

This study had 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 that include both genders and use a longitudinal design are necessary. These findings may not be generalizable to other populations, given that differences in racial attitudes toward lifestyle may influence these results. One strength of the study is that, to the best of our knowledge, it is the first study to investigate the association between GSTT1 polymorphism and DM in a sample of Yazdian females in Yazd, Iran.

5. Conclusions

The most obvious finding to emerge from this study was that the GSTT1 deletion polymorphism is associated with a greater risk of acquiring diabetes. However, we observed no significant association between the GSTT1 null genotype and DM, suggesting that the GSTT1 gene may not play a significant role in the aetiopathogeneses of DM. It is recommended that future studies investigate the role of the GSTT1 and its combination with other GST genotypes in the pathogenesis of DM and its associated complications in large-scale cohorts in a different population.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All of authors contributed to this project and article equally. All authors read and approved the final manuscript.

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