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Original Article

Association between Glutathione S-Transferase GSTM1-T1 and P1 Polymorphisms with Metabolic Syndrome in Zoroastrians in Yazd, Iran

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Abstract

Background: The aim of this study was to assess the possible association between genetic polymorphisms of the glutathione S-transferase (GST) gene family and the risk of the development of metabolic syndrome (MS) in Zoroastrian females in Yazd, Iran.

Methods: In this case-control study, GSTM1, T1, and P1 polymorphisms were genotyped in 51 randomly selected MS patients and 50 randomly selected healthy controls on February 2014 among Zoroastrian females whose ages ranged from 40 to 70 yr. DNA was extracted from peripheral blood. Data were analyzed with SPSS version 17.

Results: We observed a significant association of GSTP1-I/V (Isoleucine/ Valine) allele and GSTP1-V/V (Valine / Valine) allele with MS (P = 0.047 and P = 0.044, respectively). The combined analysis of the two genotypes, the present genotype of GSTT1, I/V and V/V alleles of GSTP1 genotype demonstrated a decrease in the risk of acquiring MS (OR = 0.246, P = 0.031). The null genotype of GSTM1, I/V, and V/V alleles of the GSTP1 genotype showed a lower risk in double combinations (OR = 0.15, P = 0.028 and OR = 0.13, P = 0.013, respectively). The combinations of the GSTM1 null genotypes and GSTT1 present genotypes and the GSTP1 I/V and V/V alleles together were associated with decreased risk of having MS in triple combinations (OR = 0.071, P = 0.039 and OR = 0.065, P = 0.022, respectively).

Conclusion: GSTP1-I/V and V/V alleles, alone or in association with GSTM1 null and GSTT1 present genotypes, are related with decreased susceptibility to the development of MS in Zoroastrian females.

Keywords: Glutathione S-transferase, Genetic polymorphism, Metabolic syndrome, Ethnic group, Iran

Introduction

Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species. There is accumulating evidence that oxidative stress is involved in various hyperglycemia-induced diabetic complications as well as insulin resistance (1–3). In recent years, there has been much interest in the role of free radicals and oxidative stress in the pathogenesis of metabolic syndrome (MS) (4, 5).

Definition of metabolic syndrome

MS is a cluster of metabolic disturbances that include abdominal obesity, atherogenic dyslipidemia (hypertriglyceridemia and/or low high-density lip-

oprotein (HDL)), elevated blood pressure, and glucose metabolism disorders, and is a determinant of cardiovascular disease and type 2 diabetes mellitus (DM) (6). In the current study, Joint Interim Statement (JIS, 2009) (6) criteria were used to identify MS. Based on JIS, MS was diagnosed when at least three of the following traits were present, i.e., : B*P* ≥ 130/85 mmHg or drug treatment for elevated BP, fasting plasma glucose (FPG) ≥ 100 mg/dl or drug treatment for elevated blood glucose, serum TG ≥ 150 mg/dl or drug treatment for elevated TG, serum HDL < 40 mg/dl in men and < 50 mg/dl in women or drug treatment for low HDL, and abdominal obesity, defined as a waist circumference (WC) in men > 89 cm and in women > 91 cm based on a study in Iran (6).

Obesity may directly induce systemic oxidative stress and that increased oxidative stress in accumulated fat is, at least in part, the underlying cause of the dysregulation of adipocytokines and the development of MS (7, 8). In addition, the imbalance between reactive oxygen species and antioxidants increases insulin resistance in mice and humans (9, 10). Glutathione S-transferases (GSTs) consist of a superfamily of dimeric phase II metabolic enzymes that catalyze the conjugation of reduced glutathione with various electrophilic compounds (11). The human GST genes are divided into four major subfamilies designated as GSTA, GSTM, GSTT, and GSTP (12). Human cytosolic GSTs have been well characterized, and they are known to be polymorphic with different polymorphism frequencies by ethnicity. The percentage of individuals who do not express the GSTM1 enzyme due to a homozygous gene deletion is higher in Caucasians and Asians than in Africans (13, 14). About 60% of Asians, 40% of Africans, and 20% of Caucasians do not express the GSTT1 enzyme (15). These homozygous gene deletions are called null genotypes. Polymorphisms of GSTM1, GSTT1, and GSTP1 have been associated with susceptibility to various forms of cancer, particularly those caused by cigarette smoking (15), resistance to chemotherapy treatment (16), and disease outcomes (17).

Recently, the GSTT1 null genotype or both the GSTT1 and GSTM1 null genotypes interacting with current-smoking status have genetic risk factor for the development of type 2 diabetes and its cardiovascular complications (18–20). 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 (21). 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 (22), thus providing an outstanding opportunity to study risk factors associated with MS in a limited genetic-variability setting.

The aim of this study was to evaluate whether GST is associated with MS and with each component of MS among the Zoroastrian female population. The rationale for the study was that the contribution of GST polymorphism to the risk of the development of MS or the components of MS is currently unknown. To the best of our knowledge, no study has yet investigated the role of GST polymorphisms and metabolic syndrome risk and its components in the Zoroastrian population of Yazd, Iran.

Materials and Methods

Study population

In this case-control study conducted at the Yazd Diabetes Research Center, 51 women with MS were selected from the subjects who participated in a community-based, cross-sectional study of the Zoroastrians living in Yazd, Iran. In this cross-sectional study done between March 2012 and March 2013 (23); 51 women met the criteria established by the JIS (6) for a diagnosis of MS, and all of them enrolled in the current study. To facilitate

equal sampling, a control group of 50 healthy women who did not met JIS's criteria for a diagnosis of MS was selected randomly from the same geographic region.

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.

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. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). 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 for all subjects to detect patients with DM. The latest criteria established by the American Diabetes Association (ADA) were used for the diagnosis of DM in the subjects (24). Blood levels of glucose, triglyceride (TG), total cholesterol (TC), 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.

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, GSTM1, and GSTP1 polymorphisms was performed using a real-time 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 primer and probe sequences are shown in Table 1. The PCR conditions were essentially the same as those described by Ko et al. (25) 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. These conditions were the same for the amplification of all three mutations. The fluorescence signal was plotted against temperature to give melting curves for each sample. Peaks were obtained at 59 °C for the Val_{105}Val and at 63 °C for the Ile_{105}Ile genotype with a double peak for the Ile_{105}Val GSTP1 genotype.

Table 1: Sequences of primers and probes used to characterize GSTT1, GSTM1 and GSTP1 polymorphisms

Gene	PCR primers	Hybridization probes
GSTT1	5′-TCCTTACTGGTCCTCACATCTC-3′	5′-LCR640-TCGAAGGCCGACCCAAGCTGGC-3′
	5′-TCCCAGGTCACCGGATCAT-3′	5′-CCGTGGGTGATGGCTGCCAAGT-FL-3′
GSTM1	5′-GAACTCCCTGAAAAGCTAAAGC-3′	5′-LCR640-ATGGCCCGCTTCCCCAGAAACTCTG-3′
	5′-GTTGGGCTCAAATATACGGTGG-3′	5′-TCACTCCTCCTTTACCTTGTTTCCTGCAAAA-FL-3′
GSTP1	5′-ACCCCAGGGCTCTATGGGAA-3′	5′-LCR640-TGTGAGCATCTGCACCAGGGTTGGGC-3′
	5′-TGAGGGCACAAGAAGCCCCT-3′	5′-TGCAAATACATCTCCCTCATCTACACCAAC-FL-3′

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, GSTM1 and GSTP1 polymorphisms 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, GSTM1 and GSTP1 genotypes in patients with MS compared to the control groups. SPSS version 17 (SPSS Inc., Chicago, Illinois, United States) was used for the analyses of the data.

Results

A total of 101 subjects (51 subjects with MS and 50 controls) were genotyped for three members of the GST family. The frequency distribution of the GSTM1, GSTT1, and GSTP1 genotypes 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.

Association between GST genotype profile and the development of metabolic syndrome

The frequencies of GSTM1, GSTT1, and GSTP1 genotypes and alleles, along with their double and triple combinations in both patients and controls, are shown in Table 2.

The frequency of the GSTM1 null genotype was 51% in the patients and 46% in the control group. Therefore, in the patients, the frequency of the GSTM1 null genotype was higher than that of the control group, but this increase was not significant (OR = 1.25, 95% CI = 0.55-2.84). The distributions of the GSTT1 null genotypes were not significantly different between the patients and the control group (*P* = 0.98). The GSTT1 null genotype frequency was 72% in the control population and 27.5% in the patients (OR = 1.01, 95% CI = 0.41–2.40). The GSTP1-I/I (Isoleucine/ Isoleucine) allele was higher in the controls (38 vs. 17.6%) while the V/V (Valine/Valine) and I/V (Isoleucine/Valine) were higher in patients (41.2 vs. 32%) and (41.2 vs. 30%), respectively. The presence of V/V and I/V alleles was considered as low risk genotypes (OR = 0.34, 95% CI = 0.12-0.97, *P* = 0.044 and OR = 0.35, 95% CI = 0.12-0.98, *P* = 0.047, respectively).

To investigate further if GST genotypes were related to MS risk, we analyzed their association in two-way and three-way combinations to evaluate the impact of gene-gene interaction. The combination of the two genotypes, either both null (GSTM1 null/GSTT1 null) or null (GSTM1 null or GSTT1 null) showed no significant difference (OR = 0.79, 95% CI = 0.33-1.8, *P* = 0.59 and OR = 0.90, 95% CI = 0.28-2.88, *P* = 0.86, respectively). The present genotype of GSTT1, I/V and V/V alleles of GSTP1 genotype showed lower risk in double combinations (OR = 0.246, CI = 0.06-0.88, *P* = 0.031). The null genotype of GSTM1, I/V and V/V alleles of GSTP1 genotype showed lower risk in double combinations (OR = 0.15, CI = 0.02-0. 81, *P* = 0.028 and OR = 0.13, CI = 0.02-0.65, *P* = 0.013, respectively). The combinations of the GSTM1 null genotypes and GSTT1 present genotypes and the GSTP1 I/V and V/V alleles were significantly related to MS. The incidence of these two genotypes and the GSTP1 I/V and V/V alleles occurring together were associated with decreased risk of having MS (OR = 0.071, CI = 0.006-0. 8, *P* = 0.039 and OR = 0.065, CI = 0.006-0.6, *P* = 0.022, respectively). The other combined genotypes were not associated with higher or lower risk than the respective separate genotypes, however caution is required as the number of individuals carrying these genotypes in the control and patient population was relatively small.

Anthropometric and metabolic variables according to GST genotypes

We further investigated the clinical parameters accompanying high risk genotypes (GSTT1 null or GSTM1 null) compared to non-risk genotypes (GSTT1 present and GSTM1 present genotypes) in patients and controls (Table 3, 4, and 5).

Table 2: Association between GST genotype profile and the development of metabolic syndrome

Locus	Genotype	Patient (n=51) (%)	Control (n=50) (%)	Odds ratio (95 % CI)	*P*-value
GSTT1	Present	37 (72.5)	14 (28)	1.00 (Ref)	
	null	14 (27.5)	38 (72)	1.01 (0.41-2.40)	0.98
GSTM1	Present	25 (49)	27 (54)	1.00 (Ref)	
	null	26 (51)	23 (46)	1.25 (0.55-2.84)	0.59
GSTP1	I/I	9 (17.6)	19 (38)	1.00 (Ref)	
	I/V	21 (41.2)	15 (30)	0.35 (0.12-0.98)	0.047
	V/V	21 (41.2)	16 (32)	0.34 (0.12-0.97)	0.044
Two loci GSTT1and GSTM1	Both present	19 (37.3)	21 (42)	1.00 (Ref)	
	Either null	24 (47.1)	21 (42)	0.90 (0.28-2.88)	0.86
	Both null	8 (15.7)	8 (16)	0.79 (0.33-1.8)	0.59
GSTT1 (+/+) and GSTP1	T1 (+/+) and I/I	5 (13.5)	14 (38.9)	1.00 (Ref)	
	T1 (+/+) and I/V	16 (43.2)	11 (30.6)	0.246 (0.06-0.88)	0.031
	T1 (+/+) and V/V	16 (43.2)	11 (30.6)	0.246 (0.06-0.88)	0.031
GSTT1 (-/-) and GSTP1	T1 (-/-) and I/I	4 (28.6)	5 (35. 7)	1.00 (Ref)	
	T1 (-/-) and I/V	5 (35. 7)	4 (28.6)	0.8 (0.13-4.8)	0.809
	T1 (-/-) and V/V	5 (35. 7)	5 (35. 7)	0.64 (0.1-4.1)	0.63
GSTM1 (+/+) and GSTP1	M1 (+/+) and I/I	6 (24)	8 (29.6)	1.00 (Ref)	
	M1 (+/+) and I/V	7 (28)	8 (29.6)	0.68 (0.18-2.6)	0.58
	M1 (+/+) and V/V	12 (48)	11 (40.7)	0.85 (0.19-3.7)	0.85
GSTM1 (-/-) and GSTP1	M1 (-/-) and I/I	3 (11.5)	11 (47.8)	1.00 (Ref)	
	M1 (-/-) and I/V	14 (53.8)	7 (30.4)	0.15 (0.02-0.81)	0.028
	M1 (-/-) and V/V	9 (34.6)	5 (21.7)	0.13 (0.02-0.65)	0.013
Three loci M1 (+/+), T1 (+/+) and GSTP1	M1 (+/+), T1 (+/+) and I/I	4 (21.1)	7 (33.3)	1.00 (Ref)	
	M1 (+/+), T1 (+/+) and I/V	5 (26.3)	6 (28.6)	0.45 (0.09-2.1)	0.319
	M1 (-/-), T1 (+/+) and V/V	10 (52.6)	8 (38.1)	0.68 (0.12-3.7)	0.66
M1 (-/-), T1 (+/+) and GSTP1	M1 (-/-), T1 (+/+) and I/I	1 (5.6)	7 (46.7)	1.00 (Ref)	
	M1 (-/-), T1 (+/+) and I/V	11 (61.1)	5 (33.3)	0.071 (0.006-0.8)	0.039
	M1 (-/-), T1 (+/+) and V/V	6 (33.3)	3 (20)	0.065 (0.006-0.6)	0.022
M1 (+/+), T1 (-/-) and GSTP1	M1 (+/+), T1 (-/-) and I/I	2 (33.3)	1 (16.7)	1.00 (Ref)	
	M1 (+/+), T1 (-/-) and I/V	2 (33.3)	2 (33.3)	3 (0.15-59)	0.472
	M1 (+/+), T1 (-/-) and V/V	2 (33.3)	3 (50)	2 (0.9-44)	0.661
M1 (-/-), T1 (-/-) and GSTP1	M1 (-/-), T1 (-/-) and I/I	2 (25)	4 (50)	1.00 (Ref)	
	M1 (-/-), T1 (-/-) and I/V	3 (37.5)	2 (25)	0.33 (0.02-3.9)	0.38
	M1 (-/-), T1 (-/-) and V/V	3 (37.5)	2 (25)	0.33 (0.02-3.9)	0.38

n: number of sample

In the cases of GST1 null genotypes, there were higher levels of triglycerides and HDL compared to the GSTT1 present genotype ($P = 0.02$ and $P = 0.049$, respectively) in the controls. In patients, there were higher levels of LDL in GSTT1 present genotype compared to the GSTT1 null genotype ($P = 0.031$) (Table 3). In cases of the GSTP1-I/I allele, patients had higher WC and BMI compared to the V/V and I/V ($P = 0.046$ and $P = 0.004$, respectively). There were no significant differences in other components in each of genotypes.

Table 3: Anthropometric and metabolic variables according to GSTM1 genotype

Clinical parameters	Case			Control		
	Present (n=25)	Null (n=26)	*P-value*	Present (n=27)	Null (n=23)	*P*-value
Age (yr)	63±9.1	60±9.6	0.188	51.6±9.8	54.08±12.8	0.448
TG (mg/dl)	181.7±73.4	161.9±73.1	0.34	151.7±56.9	160.9±49.07	0.546
TC (mg/dl)	195.6±33	200.5±36.8	0.62	205.4±42.06	208.1±39.4	0.817
LDL (mg/dl)	118.1±22.3	124.9±26	0.323	128.5±25.4	136.3±19.7	0.237
HDL (mg/dl)	35.9±6.9	40.7±11.1	0.069	40.7±7.8	40±8.6	0.764
SBP (mmHg)	127±13.2	127.6±18.4	0.879	117.5±13.2	120±20.6	0.621
DBP (mmHg)	79.8±7.1	78.6±9.2	0.623	74.4±7.1	74.7±9.3	0.885
WC (cm)	95.5±8.7	91.9±11.5	0.216	88.8±9	88.6±10.5	0.941
WHR	0.89±0.04	0.87±0.06	0.153	0.86±0.06	0.86±0.07	0.947
BMI (kg/m^2)	26.9±4	26.3±4.3	0.629	25.5±3.2	24.9±4.06	0.574

Data are reported as means ± S.D.; n: number of sample; TG: triglyceride; TC: total cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; WHR: waist to hip ratio; BMI: body mass index

Table 4: Anthropometric and metabolic variables according to GSTT1 genotype

Clinical parameters	Case			Control		
	Present (n=25)	Null (n=26)	*P*-value	Present (n=36)	Null (n=14)	*P*-value
Age (yr)	61.8±9.9	61.4±8.6	0.892	51.5±11.2	56±12.5	0.209
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
SBP (mmHg)	127.4±15.7	127.1±17	0.955	119.1±15.8	117.5±19.9	0.758
DBP (mmHg)	79±8.4	79.6±7.7	0.882	74.5±7.3	74.6±10.2	0.982
WC (cm)	93±10	95.6±11.1	0.419	89.6±9.2	86.4±10.7	0.3
WHR	0.88±0.05	0.88±0.05	0.747	0.87±0.06	0.85±0.07	0.33
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; TG: triglyceride; TC: total cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; WHR: waist to hip ratio; BMI: body mass index

Table 5: Anthropometric and metabolic variables according to GSTP1 genotype

Clinical parameters	Case				Control			
	I/I (n=9)	I/V (n=21)	V/V (n=21)	*P-value*	I/I (n=19)	I/V (n=15)	V/V (n=16)	*P*-value
Age (yr)	62±8.1	62.7±9.9	60.6±9.8	0.778	54.1±10.8	52.3±10.3	51.5±13	0.781
TG (mg/dl)	183.6±56.1	169.9±78.7	168.2±76.6	0.866	24.6±4	153.3±46.3	147.6±69.1	0.622
TC (mg/dl)	196.8±33.8	191.8±33.2	205±37	0.471	207.4±38.5	212.6±39.5	200.3±45	0.703
LDL (mg/dl)	117.2±26	118.7±22.6	126.4±25.5	0.502	136.2±23.3	133.6±20.3	125.8±25.2	0.404
HDL (mg/dl)	38.5±8.4	38.5±9.5	38.1±10.4	0.988	39.3±9.1	42.8±6.7	39.2±8	0.375
SBP (mmHg)	133.3±16.5	128.5±12	123.5±18.6	0.282	119.2±17.6	124.6±12.4	112.5±18.3	0.132
DBP (mmHg)	80±8.2	81.4±6.3	76.6±9.3	0.164	76±9.2	76.3±8.1	71.2±5.9	0.135
WC (cm)	99.8±10.7	94.7±11.1	90±7.8	0.046	87.6±8.5	87.8±10.2	90.8±10.5	0.585
WHR	0.89±0.06	0.88±0.05	0.87±0.05	0.571	0.85±0.07	0.87±0.07	0.87±0.06	0.742
BMI (kg/m^2)	30.3±5.8	26.7±3.2	25±3.1	0.004	24.6±4	24.9±3.2	26.3±3.4	0.352

Data are reported as means ± S.D.; n: number of sample; TG: triglyceride; TC: total cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; WHR: waist to hip ratio; BMI: body mass index

Discussion

In this case-controlled study, GSTM1-T1 and P1polymorphisms were evaluated for its association with susceptibility to MS. Our study showed that GSTP1-I/V and V/V alleles, alone or in association with GSTM1 null and GSTT1 present genotypes, were related with decreased susceptibility to the development of MS in Zoroastrian females. Considering MS, a cluster of risk factors (visceral obesity, insulin resistance, dyslipidemia, and hypertension) is increasingly being recognized as a new risk factor for type 2 diabetes and atherosclerotic cardiovascular disease (26). DM is associated with an increased production of reactive oxygen species and a reduction in antioxidant defenses. Studies have shown that individuals with lowered antioxidant capacity are at increased risk of diabetes (27, 28). Members of the GST supergene family are critical for protecting cells from reactive oxygen species (ROS) because they can utilize a wide variety of products of oxidative stress as substrates (29). GSTT1 utilizes oxidized lipids and oxidized DNA; GSTP1 catalyzes the detoxification of products that arise from DNA oxidation (15). The lack of detoxification, which is genetically determined, may be a risk factor for the development of diabetes. Therefore, analysis of GST gene status, particularly detection of GSTM1 and GSTT1 null mutations and GSTP1 polymorphism, could have clinical importance.

This study analyzed the GSTT1, GSTM1, and GSTP1 polymorphisms in association with MS in 51 patients and 50 controls from an ethnic minority group (Zoroastrian) in Yazd, Iran. In our study, no significant differences in the frequencies of the GSTT1 and GSTM1 null mutations were observed between the patients and the control group. This frequency was in accordance with Yalin et al. (30). We may state that GSTT1 and GSTM1 polymorphism does not influence the risk of MS in the female Zoroastrian population. Many studies have dealt with GST polymorphism in various diseases, but only a few studies have addressed the role of GST polymorphisms in MS. Kuzuya et al. reported that there was an association between glutathione peroxidase 1 (GPX1) 198Leu variants and central obesity in men (31). They also reported that CT/TT genotypes were associated with the higher prevalence of metabolic syndrome in men. Therefore, they speculated that these associations suggested that a weaker antioxidant defense system and greater oxidative stress might be causative factors for obesity. In addition to GPX1, it was reported that defective glutathione peroxidase 3 (GPX3) expressions in adipose tissue is associated with reduced systemic GPX activity and increased oxidative stress in obesity (32). Lee et al. proposed that local ROS accumulation in the adipose tissue of obesity could be expanded into systemic oxidative stress by the vicious cycle wherein increasing local ROS accumulation suppresses adipose GPX3 expression (32). Park et al. performed a study on Korean schizophrenic patients to investigate the polymorphism of GST's and olanzapine-induced weight gain. They found that there was no difference in the null genotype distribution of GSTM1 and GSTT1 between subjects with body weight gain ≥7% compared to subjects with body weight gain <7% ($P > 0.05$). No significant difference in GSTP1 genotype and allele frequencies were observed between the groups ($P > 0.05$) (33). In the current study, the GSTP1- I/V and V/V alleles appear to have a protective effect against the development of MS. When the effect of combined genotypes is different from the sum of the independent effects of each genotype, there is a possible interaction or synergistic effect of each genotype. In our study, the risk of MS was decreased in subjects who had a combination of the GSTT1 present genotype and the GSTP1- I/V and V/V allele, and the risk decreased in a combination of the GSTM1 null and the GSTP1- I/V and V/V genotype. When we investigated three genotype combinations, the risk decreased in combinations of GSTM1 null and GSTT1 present and the GSTP1- I/V and V/V allele. These data indicate that the presence of the combined GSTM1 null, GSTT1 present, and GSTP1- I/V and V/V allele have a protective role for decreased susceptibility to MS. Yalin et al. found that a combination of the GSTM1 null genotype and GSTT1 present genotype and the

GSTP1 Val allele increased the risk of DM four-fold in subjects with DM. They also indicated that the presence of the combined GSTM1 null, GSTT1 null, and GSTP1 Ile/Ile genotypes is a risk factor for enhanced susceptibility to diabetes (30).

In this study, we found that the association between LDL level and GSTT1 genotype was statistically significant and that subjects with the GSTT1 present genotype had higher level of LDL than those with the null genotype. We also showed that there was a significant relationship between WC and BMI in GSTP1 genotypes, while subject with I/I allele had higher WC and BMI. The findings of the present study are in agreement with a study conducted by Amer et al. showing that patients with the GSTT1 null genotype had higher levels of triglycerides and very low-density lipoprotein cholesterol than those with the GSTT1 present genotype. They also showed that patients with the GSTM1 null genotype had significantly higher levels of HbA1c and significantly higher diastolic blood pressure than those with the GSTM1 present genotype (34).

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 GSTM1, GSTT1, and GSTP1 polymorphisms are related with MS risk, 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 strength, to the best of our knowledge, this is the first study that has investigated association between GSTs polymorphisms and MS in the Zoroastrians who live in Yazd, Iran.

Conclusion

We observed that GSTP1-V/V and I/V alleles, the combination of present genotype of GSTT1, I/V and V/V alleles of the GSTP1 genotype, the null genotype of GSTM1, I/V and V/V alleles of GSTP1 genotype, the GSTM1 null genotypes, the GSTT1 present genotypes, and the GSTP1 I/V and V/V alleles had protective effects against the development of MS. These results may support the hypothesis that oxidative stress is involved in the pathogenesis of MS. These results warrant further investigation in large-scale cohorts in different populations to confirm the role of GSTM1, T1, and P1 gene polymorphisms in the pathogenesis of MS and its associated complications.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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