Glutathione S-Transferase M1 and T1 Gene Polymorphisms Are Not Associated with Increased Risk of Gestational Diabetes Mellitus Development

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ABSTRACT

Aim: *The aim of this study was to investigate whether the glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) gene polymorphisms contributed to development of gestational diabetes mellitus (GDM).*
Subjects and Methods: *Fifty women with diagnosis of GDM and 50 control individuals without GDM or altered glucose intolerance during their pregnancy were enrolled in the study. Multiplex polymerase chain reaction-restriction fragment length polymorphism method was applied to determine the GSTM1 and GSTT1 gene polymorphisms. Genotypes were determined according to bands detected with the agarose gel electrophoresis.*
Results: *The difference in the frequencies of GSTM1 null genotypes between GDM and control groups was not statistically significant (60% and 54%, respectively). There was no statistically significant difference between GDM and control groups with respect to GSTT1 null genotype rates (22% and 20%, respectively).*
Conclusion: *This study shows no association between GST gene polymorphisms and GDM.*

Keywords: Gene, gestational diabetes mellitus, glutathione S-transferase, GSTM1, GSTT1, polymorphism

Los Polimorfismos de Genes de la Glutación S-transferasa M1 y T1 No Están Asociados con el Aumento de Riesgos en el Desarrollo de la Diabetes Mellitus Gestacional

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RESUMEN

Objetivo: *El objetivo de este estudio fue investigar si los polimorfismos de genes de la glutación s-transferasa M1 (GSTM1) y la glutación s-transferasa T1 (GSTT1) contribuyen al desarrollo de la diabetes mellitus gestacional (DMG).*
Materiales y Métodos: *Cincuenta mujeres con diagnóstico DMG, y otras 50 sujetos de control sin DMG o intolerancia a la glucosa alterada durante el embarazo, fueron reclutadas para este estudio. Se aplicó el método de reacción en cadena de la polimerasa (RCP) múltiple-polimorfismo de longitud de fragmentos de restricción, con el objeto de determinar los polimorfismos genéticos de GSTM1 y GSTT1. Los genotipos fueron determinados según las bandas detectadas con la electroforesis en gel de agarosa.*
Resultados: *La diferencia en las frecuencias de los genotipos nulos GSTM1 entre los grupos DMG y control, no fue estadísticamente significativa (60% y 54%, respectivamente). No hubo ninguna diferencia estadísticamente significativa entre los grupos control y DMG con respecto a las tasas de genotipo nulo GSTT1 (22% y 20%, respectivamente).*
Conclusión: *El estudio no muestra asociación alguna entre los polimorfismos genéticos de GST y DMG.*

Palabras claves: Gen, GSTM1, GSTT1, diabetes mellitus gestacional, glutación s-transferasa, polimorfismo

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INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the major medical problems that affect the mother and the fetus during pregnancy. It is observed in 1 to 14% of pregnancies, whereas the incidence of pregestational diabetes is 0.5% (1, 2). Gestational diabetes mellitus is usually encountered after the 24th week of the pregnancy, as a result of the effects of the placental hormones on the maternal glucose metabolism. A misdiagnosis or underestimation in the diagnosis of the disease during pregnancy causes an increase in perinatal morbidity and mortality (3, 4). It is therefore important to define the population that is at risk of developing GDM.

It is vital for the organism to maintain antioxidant capacity for the continuity of the cellular stability and vitality. Oxidative stress is suggested to contribute to the physiological processes in ageing and pathological processes in many diseases such as diabetes, atherosclerosis and cancer (5–7). The glutathione S-transferase (GST) enzymes which are involved in phase II detoxification reactions are a member of the dimeric enzyme family. They play an important role in detoxifying cytotoxic agents, and protect cellular macromolecules (8, 9). Although GST enzymes are involved in conjugation of a wide variety of suspected carcinogens including aliphatic aromatic heterocyclic radicals, epoxides and arene oxides, their main function is the provision of the cellular defence against oxidative stress by conjugation of reactive oxygen species with gluthathione (10). Based on the structural and biochemical characteristics, seven classes of GST enzymes covering alpha, mu, omega, pi, sigma, theta and zeta subclasses were identified (11). Among them, mu (coded from GSTM1) and theta (coded from GSTT1) enzymes are preferentially studied because of their potential to modulate individual vulnerability to oxidative stress. Glutathione S-transferase genes have polymorphic variants that affect the activity and amount of the GST enzymes, and therefore result in diminished cellular resistance (12).

A grave reduction in the activity of GSTM1 and GSTT1 enzymes is due to the homozygous deletion of these genes [a null genotype] (13, 14). It was shown that the GSTM1 gene is non-functional in 35% to 60% of the human race, and in almost 50% to 60% of the Caucasian population (15, 16). In the United States of America (USA), it is non-functional in 23% to 41% of persons of African descent (17). Similarly, the GSTT1 gene is also polymorphic, and non-functional in 10% to 65% of the diverse human populations. Seventeen per cent of the American Caucasians, 38% of the Malaysians, and 3.2% of the Britons living in India do not have functional GSTT1 genotype (17, 18). Among the populations, Asians have the highest reported GSTT1 null genotype.

The relationship between polymorphisms in GST genes and diabetes mellitus (DM) has been investigated in a limited number of studies (19–21). Moreover, to the best of our knowledge, there was no study researching the effect of polymorphisms in GST genes as a risk factor for GDM development. We thought that polymorphisms in the GSTM1 and GSTT1 genes might alter cellular defence against oxidative stress, and thus, increase the risk of GDM development.

SUBJECTS AND METHODS

This study was conducted as a prospective case-control study between April 2009 and May 2010. The study group consisted of 50 patients with a recent diagnosis of GDM. Fifty healthy age-matched participants without any history of GDM were admitted to serve as the control group. Both groups were recruited during the same time period and were from the same geographic region. Volunteers with chronic hepatic or renal diseases, multiple pregnancies and overt diabetes mellitus were excluded from the study. Criteria with regard to serum glucose measurements according to the National Diabetes Data Group (NDDG) were agreed in the study.

A cut-off value at 50 grams glucose challenge test (GCT) for the further analysis of the patient was defined as 140 mg/dL, as suggested by the American Diabetes Association and American Congress of Obstetricians and Gynecologists (4). A cut-off value at 50 grams GCT for the definite diagnosis of GDM was defined as 200 mg/dL. Patients whose serum glucose levels were measured in normal ranges at 100 grams oral glucose tolerance test (OGTT) survey, although they exhibited higher glucose levels in 50 grams GCT (>140 g/dL), were excluded from the control group. Members of both the study and control groups were informed and asked to sign a written consent form. Demographic characteristics including age, body mass index (BMI), gravidity, parity, smoking habit and alcohol use of the participants and family history of diabetes mellitus were recorded. Body mass index was calculated as weight (in kilograms) divided by the square of the height (in metres). The study was approved by the Ethics Committee of Uludag University School of Medicine, and all participants signed an informed consent form.

DNA extraction and GST genotyping

Blood samples from both the patient and control groups were taken in ethylenediaminetetraacetic acid (EDTA) containing tubes in order to isolate genomic DNA from circulating leucocytes with salting out procedure. Samples were drawn into falcon tubes and suspended with lysis buffer (approximately 6 mL) in 1:3 ratio. The cocktail was mixed-up gently for a few minutes, and incubated in +4 °C for 15 minutes. After centrifugation for 10 minutes in 1500 rpm, the supernatant was eliminated. Another 6 mL of lysis buffer was added to the precipitate, and centrifuged for 10 minutes in 1500 rpm. The supernatant was washed out again and the remaining pellet was handled with DZ® DNA isolation kit (Dr Zeydanlı Life Sciences Ltd, Turkey) according to the

manufacturer's instructions, and samples were stored at -20 °C until polymerase chain reaction (PCR) was performed. All of the DNA samples collected from participants were studied and included in the present report without any elimination.

The GSTM1 and GSTT1 polymorphisms were established by multiplex PCR method. For the GSTT1 polymorphism, forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and reverse 5'-TCA CCG GAT CAT GGC CAG CA-3' primers were used. To determine GSTM1 polymorphism, forward 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and reverse 5'-GTT GGG CTC AAA TAT ACG GTG G-3' primers were used. Albumin forward 5'-GCC CTC TGC TAA CAA GTC CTA C-3' and reverse 5'-GCC CTA AAA AGA AAA TCC CCA ATC-3' primers were used as internal controls. Polymerase chain reaction was conducted for GSTM1 and GSTT1 gene polymorphisms separately by using 3.0 µL of genomic DNA, 1.0 µL of each forward and reverse primers (10 pmol/mL), 0.3 µL of each of the dNTPs, 0.1 µL of Taq DNA polymerase (5 unit/µL), 2.5 µL $MgCl_2$, 17 µL of distilled water (dH2O), pH 8.3 in a total volume of 25 µL. Polymerase chain reaction conditions required denaturation for five minutes at 94 ºC, followed by 35 cycles of amplification as follows: one minute at 94 ºC (denaturation), one minute at 57 ºC (annealing), one minute at 72 ºC (elongation). Finally, 10 minutes at 72 ºC (final elongation) was performed. Genotypes were determined by migration of the products in agarose gel with added 2% ethidium bromide. Glutathione S-transferase T1 459 bp, GSTM1 219 bp and albumin 350 bp PCR products were produced.

Electrophoresis was conducted in 2% agarose gel to identify the genomic end products. Existence of 219 bp, 459 bp and 350 bp products was designated GSTM1, GSTT1, and albumin (control), respectively (Figure). If 219 bp or 459 bp products did not exist, then the patient was identified as null genotype for the mentioned genes.

Statistical analysis

Mean values with standard deviations were given for the descriptive variables of both groups. Statistics were performed using the Statistical Package for the Social Sciences software, version 17.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used for comparisons between the groups in terms of age, BMI, gravidity, parity, number of abortions, alcohol use, cigarette smoking and family history of diabetes mellitus. In comparison of exposed risks and genotype frequencies, Pearson's Chi-squared test and Fisher's exact test were used, as appropriate. *P*-value of < 0.05 was accepted as a statistically significant difference.

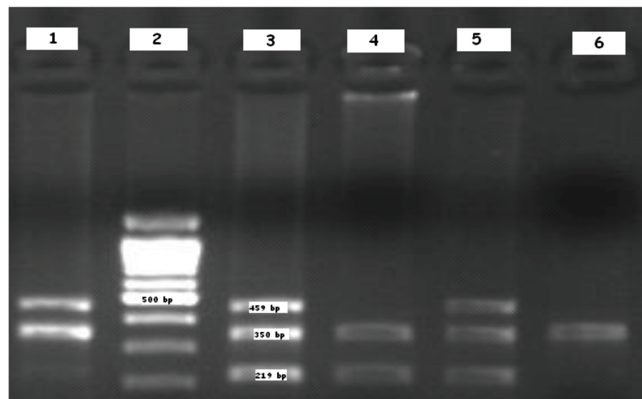

Figure: Assesment of the genotypes on agarose gel electrophoresis. Products of 219 bp, 459 bp and 350 bp were indicated on agarose gel electrophoresis after polymerase chain reaction (PCR) representing glutathione S-transferase (GST) M1, GSTT1 and albumin (control), respectively. Glutathione S-transferase T1 positivity and GSTM1 negativity was demonstrated on the first row, whereas both were positive in the 3rd and 5th rows. Both were negative in the 6th row.

RESULTS

Demographic characteristics of the study participants are given in Table 1. There was a statistically significant difference between mean ages of the patients with GDM and controls ($p < 0.01$; 32.2 ± 5.1 years and 28.6 ± 5.7 years, respectively). The difference between mean values of BMI was not statistically significant between GDM and control groups (30.2 ± 4.6 and 28.7 ± 5.0, respectively). Mean value of gravidity was significantly higher in the patients with

Table 1: Demographic characteristics of gestational diabetes mellitus (GDM) and control groups

	GDM group	Control group	Significance
Age (years)	32.2 ± 5.1	28.6 ± 5.7	**< 0.01**
Body mass index (kg/m^2)	30.2 ± 4.6	28.7 ± 5.0	0.1
GDM in previous pregnancy (%)	2%	0%	0.3
Mean arterial pressure	96.6 ± 10.9	96.6 ± 10.8	0.99
Gravidity	2.5 ± 1.6	1.9 ± 1.1	**< 0.01**
Parity	1.0 ± 1.0	1.0 ± 1.0	0.9
No. of abortions	0.8 ± 1.2	0.3 ± 0.6	**0.01**
Cigarette smoking	12%	8%	0.5
Alcohol use	0%	2%	0.3
Family history of DM	26%	10%	**0.01**
First hour blood glucose value in 50 grams glucose challenge test	187 ± 27	104 ± 16	**< 0.01**

GDM when compared with the control group ($p < 0.01$). Mean number of the abortions was significantly higher in the patient group compared to the controls ($p = 0.01$). Family history of diabetes mellitus was significantly higher in patients with GDM compared to the control group ($p = 0.01$). There was a statistically significant difference between GDM and control groups with regard to the mean value of 50 grams GCT ($p < 0.01$; 187 ± 27 and 104 ± 16, respectively).

Frequency of GSTM1 null genotype in patients with GDM was 60% (n = 30), whereas it was 54% (n = 27) in control individuals (Table 2). Glutathione S-transferase T1 null genotype in patient and control groups was 22% (n = 11) and 20% (n = 10), respectively. The differences in frequencies of both GSTM1 and GSTT1 genes between GDM and the control groups were not statistically significant ($p = 0.69$ and $p = 1$, respectively).

The difference with regard to GSTM1 genotype between participants who bore functional GSTT1 genotype in GDM and control groups was not statistically significant [$p = 0.299$, OR =1.79, 95% CI: 0.73, 4.40] (Table 3). Similarly, there was no statistically significant difference with regard to GSTT1 genotype between participants who bore functional GSTM1 genotype in GDM and control groups ($p = 0.162$, OR = 0.36, 95% CI: 0.06, 2.16). In addition, the difference between presence of both functional alleles and complete absence of them was not statistically significant ($p = 0.618$). Furthermore, absence of both functional alleles when compared with the presence of at least one functional allele was not associated with increased risk of GDM development ($p = 0.758$, OR = 0.68, 95% CI: 0.20, 2.31).

Frequencies of GSTM1 and GSTT1 null genotypes in patients with GDM who have a family history of DM were 50% and 22.2%, respectively (Table 4). The distribution of non-functional GSTM1 and GSTT1 genotypes among patients who do not have a family history of DM were 58.5% and 20.7%, respectively. Neither GSTM1 nor GSTT1 gene polymorphisms were found to be associated with family history of DM ($p = 0.69$ and $p = 1$, respectively).

The difference with regard to GSTM1 genotype between participants who bore functional GSTT1 genotype in patients with positive and negative family history of DM was not statistically significant [$p = 0.755$, OR = 0.69, 95% CI: 0.20, 2.28] (Table 5). Similarly, there was no statistically significant difference with regard to GSTT1 genotype between participants who bore functional GSTM1 genotype with positive and negative family history of DM ($p = 0.073$, OR = 0.36, 95% CI: 0.06, 2.16). Moreover, the difference between presence of both functional alleles and complete absence of them was not statistically significant ($p = 0.203$).

Table 2: Effects of glutathione S-transferase (GST) gene polymorphisms on risk of gestational diabetes mellitus (GDM) development

		GDM group n = 50	Control group n = 50	*p*	OR	95% CI
GSTM1						
	Null	30 (60%)	27 (54%)	0.69	1.28	0.58 – 2.82
	Present	20 (40%)	23 (46%)			
GSTT1						
	Null	11 (22%)	10 (20%)	1	1.13	0.43 – 2.96
	Present	39 (78%)	40 (80%)			

Table 3: Effects of specific glutathione S-transferase (GST) genotypes on risk for gestational diabetes mellitus (GDM) development

	GSTT1 (+) and GSTM1 (+) (n)	GSTT1 (+) and GSTM1 (-) (n)	GSTT1 (-) and GSTM1 (+) (n)	GSTT1 (-) and GSTM1 (-) (n)	p^1	p^2	p^3	p^4
GDM group	14	25	6	5	0.299	0.162	0.618	0.758
Control group	20	20	3	7				

(-): null genotype, (+): present genotype
p^1: significance of the statistical analysis between participants with GSTT1 (+) and GSTM1 (-) and GSTT1 (+) and GSTM1 (+) genotypes
p^2: significance of the statistical analysis between participants with GSTT1 (-) and GSTM1 (+) and GSTT1 (+) and GSTM1 (+) genotypes
p^3: significance of the statistical analysis between participants with GSTT1 (-) and GSTM1 (-) and GSTT1 (+) and GSTM1 (+) genotypes
p^4: significance of the statistical analysis between participants with GSTT1 (-) and GSTM1 (-) genotype and at least one copy of a functional GST genotype

Table 4: Association between family history of diabetes mellitus (DM) and gestational diabetes mellitus (GDM) in study population

		Positive familial DM history n = 18	Negative familial DM history n = 82	Significance	OR	95% CI
GSTM1						
	Null	9 (50%)	48 (58.5%)	0.69	1.41	0.50, 3.93
	Present	9 (50%)	34 (41.5%)			
GSTT1						
	Null	4 (22.2%)	17 (20.7%)	1	0.92	0.27, 3.14
	Present	14 (77.8%)	65 (79.3%)			

GSTM1: glutathione S-transferase M1, GSTT1: glutathione S-transferase T1

Table 5: Relationship of the family history of diabetes mellitus (DM) with the distribution of the glutathione S-transferase (GST) genotypes

	GSTT1 (+) and GSTM1 (+) (n)	GSTT1 (+) and GSTM1 (-) (n)	GSTT1 (-) and GSTM1 (+) (n)	GSTT1 (-) and GSTM1 (-) (n)	p^1	p^2	p^3	p^4
Positive family history of DM	5	9	4	0	0.755	0.073	0.203	0.078
Negative family history of DM	29	36	5	12				

(-): null genotype, (+): present genotype
p^1: significance of the statistical analysis between participants with GSTT1 (+) and GSTM1 (-) and GSTT1 (+) and GSTM1 (+) genotypes
p^2: significance of the statistical analysis between participants with GSTT1 (-) and GSTM1 (+) and GSTT1 (+) and GSTM1 (+) genotypes
p^3: significance of the statistical analysis between participants with GSTT1 (-) and GSTM1 (-) and GSTT1 (+) and GSTM1 (+) genotypes
p^4: significance of the statistical analysis between participants with at least one copy of a functional GST genotype and GSTT1 (-) and GSTM1 (-) genotype

Absence of both functional alleles when compared with the presence of at least one functional allele was not associated with family history of DM ($p = 0.078$).

DISCUSSION

The screening methods for gestational diabetes have not changed significantly in the last 30 years (22). The lack of widespread screening still continues in the developing countries, whereas the effectiveness of the current screening programmes and their beneficial outcomes are still a subject of discussion in developed countries where these programmes have been applied for several years. The substantial rate of GDM complications in developed countries, despite the screening programmes and follow-up that are carried out in antenatal care, has led to intense questioning of the validity of the current screening methods. Moreover, there are studies which claim that screening, diagnosis and the treatment of GDM reduce serious perinatal morbidities and mortality, but do not ensure universal health (22, 23). The basic disadvantage of the current screening methods is not the sensitivity or the competency of the 50 grams GCT or the 100 grams OGTT, but the timing of screening and the limited time interval in which the treatment should be done. As mentioned previously, the screening at the end of the second trimester, followed by 100 grams OGTT, diet and postprandial blood glucose monitoring phases take a lot of time. Consequently, the remaining time interval for the ideal treatment is very limited. Therefore, the discrimination of patients who are at risk of developing GDM will provide more effective patient management and perinatal outcome.

Oxidative stress has been proposed as one of the pathogenetic factors in the development of DM and its complications (12, 24). As the GST gene family has an important role in protection from oxidative stress, inadequecy of detoxification mechanisms may be one of the risk factors in the development of DM and GDM. This supports the clinical importance of investigating the individual GST gene status. Although there are many studies investigating the gene polymorphisms of GST in various diseases, a limited number of studies concerning the relation of DM with the GST gene polymorphism have been conducted. To the best of our knowledge, there is no study investigating the relationship between polymorphisms of GST gene family with respect to GDM so far.

Yalin *et al* have found that the frequency of GSTM1 null genotype was significantly higher in patients with DM when compared with the control group [64.3% and 32.7%, respectively] (19). In the same study, the GSTT1 and GSTP1 were suggested to have no effect, unlike the GSTM1 polymorphism, on the development of DM. However, in our study, there was no statistically significant difference in GSTM1 null genotype between the patients with GDM and control group. Additionally, we found that GSTT1 null genotype was also not associated with development of GDM.

In the present study, we further investigated the possible contribution of specific GST genotypes for predisposition to GDM development. We found that presence of non-functional alleles for any of the GSTM1 and GSTT1 genes did not influence GDM. Interestingly, even the presence of at least one functional GST allele did not protect against the occurrence of the disease. Similarly, Fujita *et al* investigated the GSTM1 polymorphisms in patients with Type II diabetic nephropathy (20). The GSTM1 null genotype was determined at a rate of 48.6% in patients with nephropathy, while the rate was 55.1% in those without nephropathy. The authors concluded that the GSTM1 null genotype did not contribute to the development of diabetic nephropathy. The results of the study by McRobie *et al* supported ours; they investigated the placental GST enzyme activity in patients with overt diabetes, GDM and controls who were in their 34th to 41st weeks of gestation (25). The authors reported a significantly decreased enzyme activity in the overt diabetes group, whereas there was no difference in enzyme activity between control and GDM groups.

Family history is important in the patients with Type II diabetes with a coexistence of 100% in monozygotic twins. Forty per cent of the siblings and one-third of the children develop abnormal glucose tolerance or overt diabetes. If both parents are diabetic, this ratio increases up to 60–75%. In GDM, in which there is an insulin resistance in target tissues, a familial predisposition is also present (26, 27). In our GDM group, the family history of DM was more common than in the control group (26% and 10%, respectively), but the difference did not reach statistical significance. Moreover, there was no statistical difference in GSTM1 and GSTT1 null genotypes between patients with a family history of DM and patients without a family history in our study population (50% *vs* 58.5%, and 22.2% *vs* 20.7%, respectively). Similar to our previous results, there was no special contribution of the GST genotypes on the presence of a family history of DM. The significant age difference and lack of examination of the GSTP1 gene polymorphism status between GDM and control groups, and the small number of cases were limitations of this study. In this respect, the outcomes of this study need to be confirmed.

This is the first study to investigate the relationship between GDM and GST gene polymorphisms. Glutathione S-transferase M1 and GSTT1 gene null polymorphisms were not found at a higher rate in patients with GDM when compared with control individuals.

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