Glutathione S-Transferase Gene Polymorphisms and the Development of New-Onset Diabetes After Liver Transplant

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Abstract

Objectives: The association between the glutathione S-transferase polymorphisms and the development of new-onset diabetes mellitus after liver transplant was studied.

Materials and Methods: Peripheral blood samples were collected from 106 liver transplant patients divided into 2 groups: 52 with new-onset diabetes mellitus and 54 without new-onset diabetes mellitus; 169 healthy individuals with no clinical evidence of diabetes mellitus were selected as a control group. The multiplex polymerase chain reaction technique was used for genotyping GSTM1 and GSTT1 genes, using the cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) gene as an internal control. The genotype of GSTP1 was determined using the restriction fragment length polymorphism-polymerase chain reaction technique.

Results: The frequency of both GSTM1 null and GSTT1 null genotypes was not significantly different in liver transplant patients with new-onset diabetes mellitus compared with the control group (P = .11 for GSTM1; P = .71 for GSTT1). Also, there was no statistically significant association between the frequency of the GSTP1 genotypes in the liver transplant patients with new-onset diabetes mellitus compared with controls. Neither GSTM1 nor GSTT1 null genotypes were associated with the risk of developing new-onset diabetes mellitus (P = .22 for GSTM1; P = .56 for GSTT1). However, the frequency of the heterozygous mutation (AG) in the A313G GSTP1 polymorphism in patients with new-onset diabetes mellitus was significantly higher than in patients without new-onset diabetes mellitus (55.8% vs 7.4%; P = .00). Thus, the risk of developing new-onset diabetes mellitus was significantly higher in patients presenting with heterozygous GSTP1 genotypes (odds ratio = 15.76; 95% confidence interval = 4.53-60.28; P = .00).

Conclusions: The GSTP1 AG genotype was associated with an increased susceptibility to the development of new-onset diabetes mellitus after liver transplant.

Key words: Alleles, Genetic polymorphisms, Genotyping techniques, Graft survival, Hyperglycemia, Oxidative stress, Pancreatic beta cells, Type 2 diabetes mellitus

Introduction

New-onset diabetes mellitus (NODM) or post-transplant diabetes mellitus is a metabolic disorder that occurs in approximately 15% of liver transplant recipients without any previous history of hyperglycemia.1,2 However, the effects of post-transplant diabetes mellitus on patient survival have been controversial. For example, NODM has been demonstrated to impact patients’ quality of life and possibly to impair graft survival as well as to increase the possibility of infections, neurologic complications, and cardiac events.1,3-6

It appears that both genetic and environmental factors are involved in the pathogenesis of NODM.7 In large part, the pathophysiology of NODM combines pancreatic β-cell dysfunction with the presence of insulin resistance.8-10 β-Cell dysfunction and hyperglycemia in turn create a vicious cycle that aggravates the condition.8-10

In transplant patients, using immunosuppressive agents such as corticosteroids and calcineurin inhibitors (ie, cyclosporine and tacrolimus) lowers the risk of organ rejection and increases allograft survival.11 However, the majority of these agents are diabetogenic because they either reduce β-cell function or induce central or peripheral insulin resistance.8,10
The glutathione S-transferase (GST) enzymes play a key role in the inactivation of endogenous end-products produced during oxidative stress through the conjugation of glutathione (GSH), a scavenger peptide, with electrophilic compounds. Post-transplant hyperglycemia and elevated plasma free fatty acids (FFAs) lead to increased plasma glucose and FFA oxidation and, consequently, to reactive oxygen species generation through the respiratory electron transport chain. These events are followed by a concomitant decrease in mitochondrial nicotinamide adenine dinucleotide phosphate and GSH. Glutathione is important for reactive oxygen species detoxification by glutathione peroxidase. This process leads to a decrease in adenosine triphosphate and hyperpolarization of adenosine triphosphate-sensitive channels that prevent calcium dependent insulin secretion. Diabetes results in decreased expression of GSH-dependent antioxidant enzymes such as GST and glutathione peroxidase. The outcome of GSH depletion is FFA and reactive oxygen species-mediated apoptosis in β cells.

Hyperglycemia induces apoptosis by activation of nuclear factor kappa-beta, mitochondrial cytochrome C, and overexpression of the apoptotic genes. In addition, increased FFA induces ceramide and nitrous oxide formation and aggravated β-cell apoptosis. Finally, high glucose levels possibly induce apoptosis via the upregulation of Fas-to-Fas-ligand in human islets. In contrast, insulin administration has been shown to increase GST gene expression through the PI3K/AKT pathway that activates mTOR (mechanistic target of rapamycin) and to reduce intracellular oxidative stress.

It has been demonstrated that polymorphisms in the GST gene may impair the capacity to defend against oxidative stress and lead to the development of diabetes mellitus. Three of the GST genes—GSTT1, GSTM1, and GSTP1—are functional polymorphisms that lead directly to changes in the quantity or activity of their encoded enzymes in the general population.

GSTT1 and GSTM1 are the most common polymorphisms of GST enzymes having major ethnic differences in human populations, and they have been studied the most extensively. Deletion polymorphisms of the GSTT1 and GSTM1 genes in homozygote subjects (known as the null genotypes) lead to a complete lack of phenotypic activity of enzyme isoforms. A 50-kb deletion occurs in the GSTT1 null genotype, which has frequencies of 11% to 38% in different populations, whereas a 15-kb deletion occurs in the GSTM1 null genotype, which has a population frequency of 20% to 70%.

The transition of A→G at DNA nucleotide 313 (A313G) is known as the polymorphic site of the GSTP1 gene that leads to isoleucine→valine substitution at codon 105 (isoleucine [Ile] 105Val) in exon 5 during translation. This substitution results in 3 GSTP1 genotypes, including the Ile/Ile homozygous wild type, valine/valine (Val/Val) homozygous variant, and heterozygote (Ile/Val) variant. The thermal stability and enzyme activity of GSTP1 are decreased in the variant homozygote.

In the present study, we determined the genotype frequency of the GSTM1, GSTT1, and GSTP1 polymorphisms in liver transplant recipients and their association with the risk of NODM after transplant in a patient population in Iran.

Materials and Methods

Patient selection
This study analyzed 106 liver transplant recipients in whom transplant was performed during 2013 to 2015 at the Shiraz University of Medical Sciences, Shiraz, Iran. The ethics committee of the university approved the protocol, which conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all patients. The inclusion criteria were no previous diagnosis of diabetes, pretransplant fasting plasma glucose level < 5.5 mmol/L, and undergoing follow-up for ≥ 10 months. The exclusion criteria were a history of diabetes mellitus, hyperthyroidism, hypothyroidism, Cushing syndrome, or pheochromocytoma before receiving the transplant.

The patients received a treatment protocol consisting of tacrolimus, mycophenolate mofetil, and steroids. Tacrolimus was started orally at 0.1 mg/kg per day with adjusted doses to keep trough levels between 10 and 12 ng/mL in the first month posttransplant and subsequently between 8 and 10 ng/mL. Additionally, mycophenolate mofetil was given with an initial oral dose of 2.0 g/day in equally divided doses every 12 hours. Finally, methylprednisolone 1 g was given intravenously (IV) on the day of surgery along with prednisolone 20 mg, tapered to zero within 3 months. According to the American Diabetes Association criteria, NODM
was defined as a fasting plasma glucose level \( \geq 7 \) mmol/L (\( \geq 126 \) mg/dL) or a nonfasting plasma glucose level \( \geq 11.1 \) mmol/L (\( \geq 200 \) mg/dL), confirmed on at least 2 occasions, or taking antidiabetes drugs for > 1 month after transplant.

The 106 liver transplant recipients were divided into a NODM group (n = 52) and a non-NODM (n = 54) group. In addition, 169 individuals with no history of glucose intolerance were selected as the control group.

DNA extraction
Genomic DNA was isolated from buffy coat using a CinnaGen DNA extraction kit (DNP TM) (SinaClon, Tehran, Iran) according to the manufacturer’s instructions. The DNA concentration was determined by optical density at 260 nm and preserved at -80°C to perform other laboratory protocols. Restriction fragment length polymorphism-polymerase chain reaction (PCR) and multiplex PCR methods were used to determine the genotype of the GST gene in each sample (Table 1).26,27

Statistical analyses
Descriptive analyses were reported as frequencies. Quantitative variables were expressed as means ± standard deviation. For nondescriptive findings, cross-tabs and chi-square tests were carried out using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). A P value < .05 was considered statistically significant.

Results
Patient characteristics
The NODM group (52 liver transplant patients) comprised 28 men (53.8%) and 24 women (46.2%). The age range of the NODM group was between 17 and 67 years (mean, 47.5 ± 12.9 y). The non-NODM group (54 liver transplant patients) comprised 31 men (57.4%) and 23 women (42.6%) with an age range between 21 and 76 years (mean, 43.5 ± 16.7 y). The control group (n = 169) included 109 women (65%) and 60 men (35%) with an age range between 27 and 78 years (mean, 45.9 ± 10.3 y). Significant statistical differences were seen in the distributions of age and sex between the 2 study groups (P = .002 for the NODM group and P < .001 for the non-NODM group). The underlying causes of cirrhosis in the liver transplant patients are shown in Table 2.

GST genotype and allele frequencies
Tables 3 and 4 depict the genotype and allele frequencies for the GST gene in the patient and
control groups. Our results showed no significant statistical differences in the distribution of GST gene polymorphisms (GSTT1, GSTP1, GSTM1) in liver transplant patients compared with the non-NODM control group (Table 3).

Statistical analyses revealed that GSTP1 polymorphism was significantly associated with the risk of developing NODM. The frequency of GSTP1 AG was significantly higher in patients with NODM than in patients without NODM (55.8% vs 7.4%; odds ratio [OR] = 15.76; 95% confidence interval [CI] 4.53-60.28; P = .00). The AA and GG genotypes were found in 38.5% of patients with NODM and 5.8% of patients without NODM, and the calculated frequencies were 64.8% for genotype AA and 27.8% for genotype GG. However, neither GSTT1 (OR = 1.32; 95% CI 0.47-3.75; P = .56) nor GSTM1 (OR = 1.61; 95% CI 0.6-3.7, P = .22) polymorphisms were associated with an elevated risk of developing NODM (Table 4).

Discussion

New-onset diabetes mellitus is considered a common complication after liver transplant in patients without any history of diabetes mellitus. The development of NODM has been associated with an increased risk of acute graft rejection, a higher mortality rate in the first 2 years after liver transplant, and an overall decrease in quality of life.28,29

Glutathione S-transferases (GSTs) are a group of genes whose polymorphisms can impair a person’s capacity to defend against oxidative stress and increase susceptibility to chronic diseases such as diabetes.16,19,30 Several previous studies have determined the effects of GSTT1, GSTM1, and GSTP1 genotypes on

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**Table 3. Genotype and Allele Frequencies of GST Genes in All Liver Transplant Patients, Patients with NODM, and Control Group**

<table>
<thead>
<tr>
<th>GST Gene</th>
<th>Genotype or Allele</th>
<th>Patients with NODM (n = 52)</th>
<th>Control Group (n = 169)</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>Studied Liver Transplant Recipients (n = 106)</th>
<th>P Value**</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>33 (63.5%)</td>
<td>86 (50.9%)</td>
<td>.11</td>
<td>1.68</td>
<td>61 (57.5%)</td>
<td>.28</td>
<td>.76</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>19 (36.5%)</td>
<td>83 (49.1%)</td>
<td></td>
<td></td>
<td>45 (42.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>12 (23.1%)</td>
<td>35 (20.7%)</td>
<td>.71</td>
<td>1.15</td>
<td>22 (20.8%)</td>
<td>.99</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>40 (76.9%)</td>
<td>134 (79.3%)</td>
<td></td>
<td></td>
<td>84 (79.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTP1</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>20 (38.5%)</td>
<td>87 (51.5%)</td>
<td>.100</td>
<td>.59</td>
<td>55 (51.9%)</td>
<td>.94</td>
<td>.98</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>29 (55.8%)</td>
<td>75 (44.4%)</td>
<td>.15</td>
<td>1.58</td>
<td>33 (31.1%)</td>
<td>.02*</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3 (5.8%)</td>
<td>7 (4.1%)</td>
<td>.62</td>
<td>1.42</td>
<td>18 (17.0%)</td>
<td>&lt;0.01*</td>
<td>.21</td>
</tr>
<tr>
<td>Allele</td>
<td>A</td>
<td>69 (66.3%)</td>
<td>249 (73.6%)</td>
<td></td>
<td></td>
<td>143 (67.4%)</td>
<td>.11</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>35 (33.7%)</td>
<td>89 (26.4%)</td>
<td></td>
<td></td>
<td>69 (32.6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; GST, glutathione S-transferase; NODM, new-onset diabetes mellitus; OR, odds ratio

*Comparison between control population and NODM after liver transplant.

**Comparison between control population and all liver transplant recipients.

P < .05 was considered statistically significant.

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**Table 4. Allele and Genotype Distribution of GSTM1, GSTT1, and GSTP1 Genes in Liver Transplant Recipients With and Without New-Onset Diabetes Mellitus**

<table>
<thead>
<tr>
<th>GST Gene</th>
<th>Genotype or Allele</th>
<th>NODM (n = 52)</th>
<th>Non-NODM (n = 54)</th>
<th>OR (95% CI)</th>
<th>P Value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>33 (63.5%)</td>
<td>28 (51.9%)</td>
<td>1.61 (0.6-3.7)</td>
<td>.22</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>19 (36.5%)</td>
<td>26 (48.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>12 (23.1%)</td>
<td>10 (18.5%)</td>
<td>1.32 (0.47-3.75)</td>
<td>.56</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>40 (76.9%)</td>
<td>44 (81.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Recessive model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>20 (38.5%)</td>
<td>35 (64.8%)</td>
<td>0.34 (0.14-0.80)</td>
<td>.006*</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>29 (55.8%)</td>
<td>4 (7.4%)</td>
<td>15.76 (4.53-60.28)</td>
<td>.00*</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3 (5.8%)</td>
<td>15 (27.8%)</td>
<td>0.16 (0.03-0.65)</td>
<td>.002*</td>
</tr>
<tr>
<td></td>
<td>Dominant model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA + AG</td>
<td>49 (94.2%)</td>
<td>39 (72.2%)</td>
<td>6.28 (1.24-29.58)</td>
<td>.002*</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3 (5.8%)</td>
<td>15 (27.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>A</td>
<td>69 (66.3%)</td>
<td>74 (68.5%)</td>
<td>0.91 (0.49-1.68)</td>
<td>.73</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>35 (33.7%)</td>
<td>34 (31.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** GST, glutathione S-transferase; NODM, new-onset diabetes mellitus; OR, odds ratio

*Comparison between NODM and non-NODM.

P < .05 was considered statistically significant.
the development of diabetes in different ethnic groups.\cite{31,32} According to our results, no significant differences were noted in the frequency of GSTM1 null and GSTT1 null genotype polymorphisms between liver transplant patients with NODM and a control group. Our results were in accordance with data obtained from studies with statistically varied parameters.\cite{15,16,20,33} For example, Stoian and colleagues (2015) reported no significant association between the GSTM1 and GSTT1 null genotypes and the development of type 2 diabetes mellitus (T2DM) in a Romanian patient population compared with a healthy control group ($P = .96$ for GSTM1; $P = .21$ for GSTT1).\cite{23} Porojan and colleagues (2015) likewise found no increased frequency of the GSTM1 and GSTT1 null genotypes in the T2DM group compared with healthy controls (for GSTM1, OR = 1.44, CI = 0.85-2.45; for GSTT1, OR = 0.85, CI = 0.44-1.67; $P = .647$).\cite{34}

In contrast, several studies have reported a significant association between the frequency of GSTM1 null and GSTT1 null genotypes and T2DM.\cite{7,30,33} In a Turkish study,\cite{16} the GSTM1 null genotype had a higher genotype frequency in T2DM patients than in a control group and was associated with a 3-fold increased risk of T2DM (OR = 3.7, 95% CI = 2.5-6.70; $P < .05$). In a South Indian population,\cite{35} a 2-fold risk of T2DM was seen for patients with the GSTM1 null genotype (OR = 2.93, 95% CI = 2.08-4.12; $P < .001$), and a 3-fold increased risk was associated with the GSTT1 null genotype (OR = 3.11, 95% CI = 2.18-4.46; $P < .001$).\cite{35}

In our results, there was no statistically significant association between the frequency of GSTP1 genotypes in liver transplant patients with NODM compared with the control group (Table 2). Similarly, Yalin and associates (2007) and Gönlü and colleagues (2012) demonstrated the lack of any association between GSTP1 genotype polymorphisms and T2DM in Turkish patients.\cite{16,33}

We also evaluated the relation between genetic polymorphisms of GSTs and the risk of developing NODM in NODM and non-NODM liver transplant recipients. Our results showed a statistically significant association between GSTP1 genotypes and the risk of NODM development, whereas no significant correlation was found between GSTM1 and GSTT1 null genotypes and the risk of NODM development. The heterozygous (AG) mutant genotype of GSTP1 had the greatest frequency in liver transplant patients with NODM compared with non-NODM patients (55.8% vs 7.4%). This genotype was associated with a 15.8-fold increased risk of developing NODM (OR = 15.76; 95% CI = 4.53-60.28; $P = .00$) (Table 3).

Our data are consistent with those published by Bid and colleagues (2010)\cite{36} in North Indian T2DM patients and with those of Amer’s group (2012)\cite{37} and Zaki’s group (2014)\cite{15} in Egyptian T2DM patients. These studies demonstrated that the frequency of heterozygous mutant genotype GSTP1 (A313G) was significantly higher in T2DM patients than in a control group and may play an important role in the susceptibility to and risk of developing T2DM.\cite{15}

The frequency of the GSTP1 genotype was shown to be a significant risk factor for T2DM in patients with heterovariant (GSTP1 A313G) and homovariant (GSTP1 G313G) genotypes who have enhanced risk for individual susceptibility to T2DM.\cite{23,35}

**Conclusions**

Our results indicate that the AG allele of the GSTP1 (A313G) gene increases the risk of NODM after liver transplant. However, its pathogenesis needs further investigation.

**References**