Polymorphisms in the GSTT1 and GSTM1 genes are associated with increased risk of preeclampsia in the Mexican mestizo population

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ABSTRACT. Preeclampsia is a pregnancy-specific disorder in humans and a major cause of maternal and neonatal morbidity and mortality. Increasing evidence suggests that oxidative stress plays an important role in the pathogenesis of preeclampsia. The aim of this study was to investigate the relationship between null alleles of the glutathione S-transferases (GST) M1 and T1 genes and the risk of preeclampsia. This case-control study involved 112 preeclamptic and 233 normoevolutive pregnant women. The null polymorphisms were
genotyped by multiplex polymerase chain reaction (PCR). Our results showed an increased risk of preeclampsia in patients with the GSTT1 null genotype [odds ratio (OR) = 2.21; 95% confidence interval (CI) = 1.14-4.27; P = 0.018]. Our data further showed that a combination of deletion genotypes of the GSTM1 and GSTT1 genes conferred an even higher risk of preeclampsia (OR = 4.56, 95%CI = 1.59-13.09; P = 0.005). Our results provide the first evidence suggesting that a GSTT1 null polymorphism might be associated with preeclampsia in the Mexican mestizo population, and that this risk increases with the combination of both GSTT1 and GSTM1 null polymorphisms.

**Key words:** GSTM1; GSTT1; Preeclampsia

**INTRODUCTION**

Preeclampsia is a hypertensive multisystemic disorder unique to humans, which affects approximately 10% of all pregnancies with a slightly higher incidence in developing countries. Preeclampsia is a major cause of maternal deaths of obstetric cause and is responsible for high morbidity and fetal mortality (Aris et al., 2009). Preeclampsia is characterized by *de novo* hypertension (two blood pressure measurements ≥ 140/90 mmHg) and proteinuria (> 300 mg/24 h) that develops after 20 weeks of gestation in a formerly normotensive woman (Health Secretary, 2007). The pathophysiology is characterized by an abnormal vascular response to placentation that is associated with increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system, and endothelial cell dysfunction (Sibai et al., 2005).

Increasing evidence suggests that oxidative stress plays an important role in the pathogenesis of preeclampsia (Patil et al., 2009; Siddiqui et al., 2010). In this regard, superoxides and free radicals generated during pregnancy could attack lipids, proteins, and nucleic acids, resulting in damage to placental cells, tissues, and organs. In addition, maternal oxidative stress could initiate maternal vascular endothelial dysfunction and induce leukocyte activation (Orhan et al., 2003; Serdar et al., 2003).

One of the most important systems involved in the metabolism and detoxification of reactive oxygen, xenobiotics, and carcinogens, is that of glutathione S-transferases (GSTs), which catalyze the nucleophilic addition of glutathione to electrophilic centers of a wide range of substances such as aliphatic and heterocyclic radicals, epoxides, or arene oxides (Seidegard and Ekstrom, 1997).

Four families of cytosolic soluble GSTs, alpha, mu, pi, and theta, with each class consisting of one or more isoenzymes and a wide variety of substrate specificities, are known in humans, and all have been found to be genetically polymorphic. Deletion polymorphisms have been found in the GSTM1 gene (*GSTM1*) and in the GSTT1 gene (*GSTT1*) (Beckett and Hayes, 1993; Hayes and Pulford, 1995). 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 (Bailey et al., 1998; Roth et al., 2000). Approximately 60% of Asians, 40% of Africans, and 20% of Caucasians do not express the GSTT1 enzyme (Strange and Fryer, 1999).
In the present study, we aimed to investigate the relationship between null polymorphisms in \textit{GSTT1} and \textit{GSTM1} and the risk of preeclampsia.

**MATERIAL AND METHODS**

**Selection of patients**

This case-control study was approved by the Investigation Ethical Committee in the Hospital General of the Ministry of Health of Durango, Mexico in accordance with the Code of Ethics of the Declaration of Helsinki. We recruited 112 women diagnosed with preeclampsia and 233 normotensive pregnant women.

**Genotyping**

The \textit{GSTT1} and \textit{GSTM1} null polymorphisms were analyzed using multiplex polymerase chain reaction (PCR). \textit{GSTM1} and \textit{GSTT1} were amplified using the following primers: 5'-GAACCTCCCTGAAAGCCTAAGC-3' and 5'-GTTGAGCTCATAATACGTTGG-3' for \textit{GSTM1} and 5'-TTCCTTACTTGTCCTCACATCCTC-3' and 5'-TCACCCGATCATGCCCCAGCAGCA-3' for \textit{GSTT1}. As an internal control, the \textit{β-globin} gene was co-amplified using the primers 5'-ACACAACTGTTCCATAGC-3' and 5'-CAACTTTCATCCGCTTCACC-3'. Amplified DNA fragments were resolved by 3% agarose gel electrophoresis yielding 480 bp, 312 bp, and 113 bp for \textit{GSTT1}, \textit{GSTM1}, and \textit{β-globin}, respectively.

**Statistical analysis**

Independent sample Student’s \textit{t}-tests were performed using the SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA). Odds ratios (ORs) as estimates of relative risk of the disease were calculated with 95% confidence intervals (95%CIs). The ORs were adjusted for variations in age and weeks of pregnancy by means of a multivariate logistic regression model.

**RESULTS**

Table 1 shows the clinical characteristics for cases and controls. Variables that exhibited a significant difference between groups were weeks of pregnancy and systolic and diastolic blood pressure (\(P < 0.05\)). Although a significant difference between cases and controls was found with respect to weeks of pregnancy, this difference is not clinically relevant.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Cases (N = 112)</th>
<th>Controls (N = 233)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.42 (7.32)</td>
<td>24.28 (6.92)</td>
<td>0.861*</td>
</tr>
<tr>
<td>Weeks of pregnancy</td>
<td>35.16 (5.84)</td>
<td>37.51 (4.14)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>156.12 (17.81)</td>
<td>112.17 (11.64)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>100.22 (10.68)</td>
<td>70.57 (10.03)</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*Media ± Standard deviation; \(^*\)Independent sample \(t\)-test.
With regard to \textit{GSTM1} and \textit{GSTT1} null polymorphisms, we found significant differences in null genotypes between cases and controls (Table 2). For \textit{GSTM1} polymorphisms, the frequencies for the null genotype were 44.64\% and 44.2\% for cases and controls, respectively, whereas for \textit{GSTT1} polymorphisms, the frequencies for the null genotype were 18.75\% and 10.3\% for cases and controls, respectively. The \textit{GSTT1} null polymorphism showed a significant risk of preeclampsia (adjusted OR = 2.21; 95\%CI = 1.14-4.27; \( P = 0.018 \), Table 2).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (N=112)</th>
<th>Controls (N = 233)</th>
<th>OR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{GSTM1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>62 (55.35%)</td>
<td>130 (55.79%)</td>
<td>1</td>
<td>(referent)</td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>50 (44.64%)</td>
<td>103 (44.20%)</td>
<td>0.925</td>
<td>0.577-1.484</td>
<td>0.747</td>
</tr>
<tr>
<td>\textit{GSTT1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>91 (81.25%)</td>
<td>209 (89.69%)</td>
<td>1</td>
<td>(referent)</td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>21 (18.75%)</td>
<td>24 (10.30%)</td>
<td>2.21</td>
<td>1.14-4.27</td>
<td>0.018</td>
</tr>
<tr>
<td>\textit{Double genotype}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both present</td>
<td>54 (48.21%)</td>
<td>112 (48.06%)</td>
<td>1</td>
<td>(referent)</td>
<td></td>
</tr>
<tr>
<td>Either null</td>
<td>45 (40.17%)</td>
<td>115 (49.35%)</td>
<td>0.76</td>
<td>0.46-1.25</td>
<td>0.288</td>
</tr>
<tr>
<td>Both null</td>
<td>13 (11.60%)</td>
<td>6 (2.57%)</td>
<td>4.56</td>
<td>1.59-13.09</td>
<td>0.005</td>
</tr>
</tbody>
</table>

95\%CI = 95\% confidence interval; GST = glutathione-S-transferase; OR = odds ratio.

DISCUSSION

Although the exact etiology and pathogenesis of preeclampsia remain unknown, there is substantial evidence that maternal endothelial cell dysfunction and damage lead to placental hypoperfusion and ischemia. Oxidative stress has been proposed as an underlying mechanism that contributes to the endothelial dysfunction associated with preeclampsia.

\textit{GST} is a large family of anti-oxidant enzymes. Specifically, \textit{GSTT1} and \textit{GSTM1} have been associated with various pathologies including oral cancer, cervical neoplasia, breast cancer, glaucoma, hepatocellular carcinoma, and type 2 diabetes (Gao et al., 2011; Zhang et al., 2011; Song et al., 2012; Sohail et al., 2013; Yi et al., 2013; Yu et al., 2013). Moreover, several studies were previously conducted to assess the role of \textit{GST} genes in preeclampsia (Zusterzeel et al., 2000; Cetin et al., 2005; Kim et al., 2005; Zhang et al., 2008; Atalay et al., 2012; Saadat et al., 2012), but to date, only a deletion of the \textit{GSTP1} gene has been associated with the pathology (Zusterzeel et al., 2000).

This study is the first to report an association between \textit{GSTM1} and \textit{GSTT1} null polymorphisms and susceptibility to preeclampsia in the Mexican mestizo population. Pérez-Morales et al. (2008) reported that the frequencies of \textit{GSTM1}, \textit{GSTT1}, and double \textit{GSTT1/GSTM1} null polymorphisms were 0.335, 0.121, and 0.023, respectively, in a Mexican mestizo population. We found very similar results for our control group.

The results of the current study are the first to demonstrate an increased risk of pre-
eclampsia in women with the \( GSTT1 \) null genotype (OR = 2.21; 95%CI = 1.14-4.27; \( P = 0.018 \)). Furthermore, this risk was increased with the combination of both null polymorphisms (OR = 4.56; 95%CI = 1.59-13.09; \( P = 0.005 \)). The differences between our results and those previously reported may be explained on the basis of the great genetic heterogeneity in the Mexican mestizo population compared with Caucasian or Asian populations. These differences may be observed even between subpopulations from different regions throughout Mexico (Silva-Zolezzi et al., 2009). In conclusion, this is the first study to suggest that \( GSTT1 \) null polymorphisms, as well as \( GSTT1/GSTM1 \) double null polymorphisms, are associated with preeclampsia. Therefore, these polymorphisms might be a risk factor for this disorder in the Mexican mestizo population.

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