GLUTATHIONE S-TRANSFERASE M₁ AND T₁ NULL GENOTYPES, AND RISK OF BREAST CANCER

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Background – Several studies have shown that the null genotypes of glutathione S-transferase M₁ (GSTM₁) and T₁ (GSTT₁) predispose to the development of specific types of cancers. A case-control study was carried out to examine the relationship between genetic polymorphisms of GSTM₁ and GSTT₁, and breast cancer risk.

Methods – Blood samples from 57 females with breast cancer and 57 age- and sex-matched healthy individuals were collected. The eligible cases were randomly-selected patients at the Chemotherapy Unit of Nemazi Hospital, Shiraz, South of Iran from October 1999 to August 2000. Both patient and control groups were unrelated Iranian Muslims. Using polymerase chain reaction (PCR) method, the GSTM₁ and GSTT₁ genotypes were determined. The association between GSTM₁ and GSTT₁ genotypes, and the development of breast cancer was examined using odds ratio (OR) and 95% confidence intervals (CIs) derived from logistic regression analysis using SPSS 10.0 software (SPSS Inc, Chicago, IL, USA).

Results – Homozygote deletion of either GSTM₁ (OR = 1.65, 95% CI: 0.77 – 3.53) or GSTT₁ (OR = 2.07, 95% CI: 0.96 – 4.48) was not associated with a statistically significant increased risk of breast cancer, whereas deletion of both GSTM₁ and GSTT₁ genes gave rise to a statistically significant increased risk of this malignancy (OR = 4.50, 95% CI: 1.30 – 15.58). Our results revealed that the combined genotypes were highly associated with breast cancer in the less than 50-year-old patients (OR = 5.63, 95% CI: 1.32 – 24.05).

Conclusion – Our findings suggest a novel additive effect of GSTM₁ and GSTT₁ polymorphisms which plays an important role in susceptibility to breast cancer.

Keywords • breast cancer • cancer susceptibility • GSTM₁ • GSTT₁

Introduction

Breast cancer is the most common tumor in females among all registered cases of cancer in the cancer registry of Fars Province (South of Iran) and in many other populations. Although a large proportion of breast cancer cases can not be attributed to known risk factors, it is demonstrated that having a first-degree relative with breast cancer increases the risk by about 1.5 – 3.0 times. This familial association could conceivably be due to a shared environment. The much higher risk associated with some familial patterns such as having a mother or sister with bilateral breast cancer at a young age makes it more likely that familial associations have a genetic basis. Nevertheless, genetic factors are not the only explanation; breast cancer almost certainly involves a strong interaction of genetics and environment. Without the necessary environment, few will develop the cancer, but in a suitable environment, those with a genetic predisposition are in higher risk.

Glutathione S-transferases (GSTs) consist of a super-family of phase II metabolic enzymes that catalyze the conjugation of reduced glutathione with electrophilic groups of a wide variety of compounds. In general, the reactions catalyzed by GSTs are considered detoxifying and serve to protect cellular macromolecules from damage caused by cytotoxic and carcinogenic agents. Human GSTs are divided into four classes as alpha, mu, pi, and theta based on amino acid...
sequence similarity and antibody cross-reactivity.\(^3\) Human GST\(_M1\) and GST\(_T1\) are isoenzymes capable to detoxify varieties of potent environmental carcinogens including nitroso compounds and benzo(\(a\))pyrene.\(^4\)

Previous studies have shown that hereditary differences in specific GST enzyme activities are due to genetic polymorphisms.\(^3,5\) The absence of GST\(_M1\) activity is caused by inheritance of two null alleles (alleles that have a deletion of GST\(_M1\) gene).\(^5\) Similarly, individuals with no GST\(_T1\) activity also have inherited null alleles of GST\(_T1\) gene.\(^5\) Several studies have shown that the null genotypes of GST\(_M1\) \(^5\)–\(^13\) and GST\(_T1\) \(^3\), \(^10\)–\(^13\) predispose to the development of specific types of cancers.

Some studies showed that GST\(_M1\) and/or GST\(_T1\) null genotypes were associated with an increased risk of breast cancer, \(^14\)–\(^18\) whereas others did not find a significant association.\(^5\), \(^19\)–\(^22\) Thus, the relationship between the null genotypes of GST\(_M1\) and GST\(_T1\), and susceptibility to breast cancer is still an open question.

In the present study, in order to get a better insight into the association between the null genotypes of GST\(_M1\) and GST\(_T1\), and susceptibility to breast cancer, the frequencies of GST\(_M1\) and GST\(_T1\) null genotypes among Iranian breast cancer patients were determined and compared with those of healthy control group.

### Patients and Methods

#### Subjects

The eligible cases were randomly-selected patients at Chemotherapy Unit of Nemazi Hospital, Shiraz, South of Iran from October 1999 to August 2000. This case-control study included 57 female patients with pathologically confirmed primary adenocarcinoma of breast. A total of 57 healthy individuals matched with the patients in terms of age and gender were also studied as a control group. Both patient and control groups were unrelated Iranian Muslims. At the time of blood donation, participants completed a brief questionnaire that ascertained smoking status, age (age at diagnosis for breast cancer patients), alcohol consumption, and family history for malignancies. Both case and control groups had a negative history of alcohol consumption.

#### Blood sampling and extraction of DNA

Blood samples were obtained from patient and control groups. Immediately after collection, whole blood was stored at \(-20°C\) until use. Genomic DNA for polymerase chain reaction (PCR) was isolated from whole blood using thawed blood samples by standard procedure.

#### Genotyping analysis

The PCR conditions for determining GST\(_M1\) and GST\(_T1\) genotypes were the same as those reported previously.\(^3,5\) For evaluating GST\(_M1\) and GST\(_T1\) polymorphisms the amplification products were analyzed by gel electrophoresis (1.6% agarose). To test for contamination, negative controls (tubes containing the PCR mixture without the DNA template) were included in every run. A 1,030 base pair (bp) fragment was amplified by PCR with the GST\(_M1\) primers while the same was performed for a 480 bp fragment with the GST\(_T1\) primers. The absence of an amplified product was consistent with the null genotypes.\(^3,5\) Successful amplification by \(\beta\)-globin-specific primers confirmed the proper function of the PCR reaction.

This technique cannot distinguish between heterozygote and homozygote of the positive genotypes, but it conclusively identifies the null genotypes. To ensure laboratory quality control, two independent readers interpreted the gel photographs. Any sample with ambiguous results (generally due to a low PCR yield) was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

#### Statistical analysis

The association between GST\(_M1\) and GST\(_T1\) genotypes and the development of breast cancer was examined by using odds ratio (OR) and 95% confidence intervals (CIs) derived from logistic regression analysis using SPSS 10.0 software (SPSS Inc, Chicago, IL, USA). Because GST\(_M1\) and GST\(_T1\) genotypes may interact with each other in the development of breast cancer, further analysis combining the GST\(_M1\) and GST\(_T1\) genotypes was carried out. Chi-square estimation for trends of none, one, and two putatively high-risk genotype(s) was performed using Epi Info (version 6) software.

#### Results

The mean age ± SD of patients was 44.8 ± 11.1 years, with age ranging between 27 and 68 years.
The mean age ± SD of the control group was 40.3 ± 13.9 years, with age ranging between 25 and 72 years. The frequencies of GSTM1 and GSTT1 genotypes in control and patient groups are summarized in Table 1. In the control group, the frequencies of GSTM1 and GSTT1 null genotypes were 38.6 and 33.3%, respectively. The frequencies of the null genotypes of both GSTM1 and GSTT1 were 50.9% in the patient group (Table 1). The null genotype of GSTM1 was not associated with a statistically significant increased risk of breast cancer (OR = 1.65; 95% CI: 0.77 – 3.53). The GSTT1 null genotype gave a fairly significant increased risk of developing cancer (OR = 2.07; 95% CI: 0.96 – 4.48; \( p = 0.06 \)).

To investigate whether the profile of GST genotypes may be associated with the risk of breast cancer, we examined the risk of the breast cancer associated with combinations of the genotypes. The reference group consisted of individuals with two putatively low-risk genotypes, i.e. the presence of GSTM1 and GSTT1 functional alleles. Table 2 shows the risk of breast cancer associated with each combination of genotypes as well as the trend in risk associated with zero, one, and two putatively high-risk genotype(s). A significant association was observed for concurrent lack of the GSTM1 and GSTT1 active genes and susceptibility to breast cancer (OR = 4.50; 95% CI: 1.30 – 15.58).

To explore possible genes-environment interactions, we examined the association between the genotypes and breast cancer risk stratified by selected characteristics such as age at diagnosis, history of cigarette smoking and family history of breast cancer in relatives (data not shown). While statistical power to detect genes-environment interaction was limited in the present study, significant interactions were observed between double null genotypes and age at diagnosis (OR for patients less than 50 years old = 5.63, 95% CI = 1.32 – 4.05; \( p = 0.015 \)).

**Discussion**

The interindividual genetic difference(s) in susceptibility to carcinogenesis is one of the most important factors in human malignancies including breast cancer. The genetically determined differences in metabolism related to the cytochrome \( p_{50} \) and GSTs \( 3 – 21 \) have been reported to be associated with various cancer susceptibility.

Our data demonstrated that, although the GSTM1 and GSTT1 null genotypes are more frequent in breast cancer cases than in control subjects, these differences are not of statistical significance (Table 1). Our results are compatible with those of other reports.\(^{10, 15 – 17, 19 – 22}\) Polycyclic aromatic hydrocarbon diol epoxides, peroxides, N-acetyl benzoquinoneimine, estrogen, catechol metabolites of estrogen, and superoxide radicals produced by endogenous metabolism are the substrates of GSTs.\(^{25}\)

Since different GST isoenzymes are known to exhibit overlapping substrate specificities, deficiencies of GST isoenzymes may be compensated by other isoforms and utilization of alternative metabolic pathways.\(^{4, 26, 27}\) It is, therefore, anticipated to be important to determine more than one genotype to obtain a reliable picture of the potential role of metabolic polymorphisms

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>Case</th>
<th>OR</th>
<th>95% (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>Present</td>
<td>35</td>
<td>28</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>22</td>
<td>29</td>
<td>1.65</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Present</td>
<td>38</td>
<td>28</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>19</td>
<td>29</td>
<td>2.07</td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval.

**Table 1.** Association between GSTM1 and GSTT1 genotypes and development of breast cancer.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>GSTM1</th>
<th>GSTT1</th>
<th>Control</th>
<th>Case</th>
<th>OR</th>
<th>95% (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two putatively low-risk genotypes</td>
<td>Present</td>
<td>Present</td>
<td>21</td>
<td>14</td>
<td>1.0</td>
<td>(Reference)</td>
</tr>
<tr>
<td>One putatively low-risk genotype</td>
<td>Present</td>
<td>Null</td>
<td>14</td>
<td>14</td>
<td>1.50</td>
<td>(0.54 – 4.17)</td>
</tr>
<tr>
<td>Null</td>
<td>Present</td>
<td>17</td>
<td>14</td>
<td>1.24</td>
<td>(0.46 – 3.37)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>28</td>
<td>1.35</td>
<td>(0.87 – 2.32)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( p < 0.001 \) for trend for none, one, and two putatively high-risk genotypes; OR = odds ratio; CI = confidence interval.

**Table 2.** Association between GST genotypes profile and development of breast cancer.
in individual responses to environmental toxicants. Our results revealed that there is a remarkable risk of breast cancer in women with double-null GST genotype (OR = 4.50; 95% CI: 1.30 – 15.58). This finding is consistent with two reports, 10, 15 but contradicts three other ones. 9, 19, 22 Interestingly, we found that the risk increased when the two putatively high-risk genotypes were considered in women less than 50 years old (OR = 5.63; 95% CI: 1.32 – 24.05). A similar finding has also been reported. 15 This data is also consistent with the one reported. 15 This data is also consistent with the one reported. 15

Therefore, GSTM, and GSTT1 null genotypes may play an important role in younger patients similar to predisposing BRCA1 and BRCA2 genes. 30 Although the question of a possible association between alcohol and breast cancer has been controversial in recent years, substantial evidence has accumulated to support a positive association. 31, 32 The primary difference in our study population compared with others is the negative history of alcohol drinking in both case and control groups. One could speculate that the different results among the studies could be due to differences in exposures predisposing to breast cancer, the frequencies of null genotypes, and genes-environment interaction(s). However, to generalize this observation, combined genetic analysis of extended numbers of young patients with certain risk factors are needed for further study.

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References