Short Communication

Polymorphisms in the GST (M1 and T1) gene and their possible association with susceptibility to childhood acute lymphocytic leukemia in Indian population

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Glutathione S-transferases (GSTs) are enzymes involved in the detoxification of several environmental mutagens, carcinogens and anticancer drugs. GST polymorphisms resulting in decreased enzymatic activity have been associated with several types of solid tumors. We determined the frequencies of the deletion of two GST subfamilies genes, M1 and T1, in patients with acute lymphocytic leukemia (ALL). Using polymerase chain reactions, we analyzed the GSTM1 and GSTT1 genotype in 135 patients with ALL (01-10 years) and 146 age matched controls. The prevalence of GSTM1 and GSTT1 homozygous resulting in null genotypes at the GSTM1 and GSTT1 loci were detected 60 (44.44%) and 48 (35.55%) patients, and 61 (41.75%) and 23 (15.75%) controls, respectively. The presence of at least one GST deletion proved to be an independent prognostic risk factor for response to treatment. In conclusion, individuals with GSTM1 or GSTT1 deletions (or deletions of both) may have an enhanced resistance to chemotherapy and a shorter survival.

Key words: Acute lymphocytic leukemia, glutathione S-transferase, polymorphism.

INTRODUCTION

Glutathione S-transferases (GSTs) are a family of cytosolic enzymes involved in the detoxification of various exogenous as well as endogenous reactive species. GSTs function as dimers by catalyzing the conjugation of mutagenic electrophilic substrates to glutathione which are water-soluble and can be excreted from the body. In humans, 4 major subfamilies of GSTs can be distinguished and are designated as GSTα, GSTθ, GSTu, and GSTζ. Each of these subfamilies is composed of several members, some of which display genetic polymorphism. Within the GSTμ subfamily, the gene coding for GSTM1 exhibits a deletion polymorphism, which in case of homozygosity (GST M1 null) leads to absence of phenotypic enzyme activity. A similar mechanism is described for GST T1 within the GSTu subfamily, whereas the gene coding for GST P1, a member of the GSTp subfamily, displays polymorphisms within its coding region at codon 105 (Ile105Val). The coding region polymorphisms within GST P1 have been suggested to confer different catalytic activities.

Previous studies have shown that hereditary differences in specific GST enzyme activities are due to genetic polymorphisms (Pembleetal, 1994; Harada et al., 1992). The absence of GST M1 activity is caused by inheritance of two null alleles (alleles that have a deletion of GST M1 gene) (Harada et al., 1992). Similarly, individuals with no GST T1 activity also have inherited null alleles of GST T1 gene (Pemble et al., 1994). Several studies have shown that the null genotypes of GST M1 (Harada et al., 1992; Seidegard et al., 1990; Saadat et al., 2000) and GST T1 (Pemble et al., 1994) predispose to the development of specific types of cancers. Important environmental carcinogens (e.g. ben-
GST T1 and susceptibility to ALL, the frequencies of GST association between the null genotypes of GST M1 and GST T1 poly- morphisms (GST M1 and GST T1) were determined and compared with those of healthy control group.

**MATERIAL AND METHODS**

**Study group and sample collection**

The study group consisted of 135 individuals diagnosed with acute lymphocytic leukemia from various cancer hospitals in the city of Hyderabad, AP (India). Cases were children aged 1-10 years who were diagnosed with acute lymphocytic leukemia between September 2003 and May, 2005. Patients were approached, with the physician’s and ethical committee clearance. The procedures followed were in accordance with the ethical standards of responsible committee of the Institutes/Hospitals, to participate in a face-to-face interview using a structured questionnaire. For each participating case, one control was selected randomly from a list of children registered with the same local institutes who were of the same sex, year of birth (±2 years), and the study group was of Indian origin and particular region i.e. Andhra Pradesh, India. Cases diagnosed with acute lymphocytic leukemia within 6 months of being diagnosed with a prior hematomal malignancy or within one year of any other cancer were considered ineligible. This criterion also was applied to the corresponding controls of cases (pseudo diagnosis being taken as the date of case diagnosis). 135 cases were acute lymphocytic leukemia (ALL) with 142 individually matched controls and a mean age of 4.2 years.

**RESULTS AND DISCUSSION**

Both GST T1 and GST M1 were screened for individual genotypic characters assessed. These results of cases and control studies are presented in Table 1. The frequencies of GST M1 and GST T1 variants are presented in Yates corrected Chi-square and p-values. The frequency of the cases and controls in genotypic characters assessed. These results of cases and control studies are presented in Table 1. The frequencies of GST M1 and GST T1 null genotypes among Indian pediatric ALL patients were determined and compared with those of healthy control group.

**Table 1. Distribution of GST M1 and GST T1 null genotypes in ALL cases and controls.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (%) n = 135</th>
<th>Controls (%) n =146</th>
<th>OR (95% CI)</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H/H</td>
<td>75 (55.55)</td>
<td>88 (60.27)</td>
<td>1.00 (NA)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Null</td>
<td>60 (44.44)</td>
<td>61 (41.75)</td>
<td>0.87 (0.53-1.43)</td>
<td>0.36</td>
<td>0.55</td>
</tr>
<tr>
<td>GST T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H/H</td>
<td>87 (64.44)</td>
<td>123 (84.24)</td>
<td>1.00 (NA)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Null</td>
<td>48 (35.55)</td>
<td>23 (15.75)</td>
<td>0.34 (0.18-0.62)</td>
<td>14.5</td>
<td>0.00013</td>
</tr>
</tbody>
</table>

OR, crude odds ratios; CI, confidence interval with 95% of probability; NA, not applicable.

zo[a]pyrene and other polyaromatic hydrocarbons) are detoxified through the GST system. Inter-individual differences in GST enzyme activity mediated by polymorphic genes have been suggested to confer varying susceptibility to environmental cancer. However, in contrast to the role of GSTs in environmental carcinogenesis, GST genotypes conferring lower enzyme activity may be of advantage for individuals undergoing chemotherapy treatment for neoplastic disease because reduced detoxification potentially enhances effectiveness of cytotoxic drugs (Willett et al., 1989).

The major objective of this study is to assess the role of GSTs and childhood acute lymphocytic leukemia (ALL). Secondly, we aim to determine the gene frequency of these two genetic polymorphisms (GST M1 and GST T1) and to assess their roles in relation to its susceptibility to childhood cancers. In order to get a better insight into the association between the null genotypes of GST M1 and GST T1 and susceptibility to ALL, the frequencies of GST M1, and GST T1 null genotypes among Indian pediatric ALL patients were determined and compared with those of healthy control group.

**Genotyping analysis**

Blood samples were obtained from patient and control groups. Immediately after collection, genomic DNA was extraction and used for polymerase chain reaction (PCR). The PCR conditions for determining GST M1 and GST T1 genotypes were the same as those reported previously (Pemble et al., 1994; Harada et al., 1992; Stella et al., 2002). 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 bp fragment was amplified by PCR with the GST M1 primers while a 480 bp fragment was amplified with the GST T1 primers. The absence of an amplified product was consistent with the null genotypes. Successful amplification by β-globin-specific primers confirmed the proper function of the PCR reaction.

**Statistical analysis**

The association between GST M1 and GST T1 genotypes and the development of Acute Lymphocytic Leukemia was examined by using odds ratio (OR) and 95% confidence intervals (CI) derived from logistic regression analysis using EPI6 software (Epiinfo6 CDC). Because GST M1 and GST T1 genotypes may interact with each other in the development of ALL, further analysis combining the GST M1 and GST T1 genotypes was carried out.
double gene deletions. This suggests that GST M1 and GST T1 genes do play a role in regulating the effectiveness of cancer-treatment drugs. This preliminary study has shown that GSTs play a significant role in the treatment of childhood ALL. However, the data is too small for adequate analysis. The function of GSTs enzymes is mainly detoxification of xenobiotics. It may also be interesting to look at GSTs and treatment toxicity in childhood ALL, although the reverse is true that the absence of GSTs improve treatment outcome.

In conclusion, our study shows that the double gene deletions is associated with improved treatment outcome for childhood ALL and knowing a patient’s genotype for GST allows oncologists to better tailor therapy for children with ALL. There is weak correlation between the GSTs and susceptibility in childhood ALL. Our data demonstrated that, although the GST M1 and GST T1 null genotypes are more frequent in ALL cases than in control subjects, these differences are not of statistical significance (Table 1). 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 (Mannervi et al., 1988; Hates et al., 1995). 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 in individual responses to environmental toxicants.

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REFERENCES


