RESEARCH COMMUNICATION

Role of GSTM1 (Present/Null) and GSTP1 (Ile105Val) Polymorphisms in Susceptibility to Acute Lymphoblastic Leukemia among the South Indian Population

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Abstract

Acute lymphoblastic leukaemia (ALL) is the most common pediatric malignancy worldwide. The origin of this disease may be explained by a combination of genetic and environmental factors. Glutathione-s-transferases are a multi-gene family of enzymes involved in the detoxification of a wide variety of environmental carcinogens. A total of 92 immunophenotyped cases (below 25 years of age) and 150 cord blood controls were here analysed by PCR for GSTM1(Present/Null) and RQ-PCR allelic discrimination assay for GSTP1(Ile105Val). We found a significant increased risk for ALL with the GSTM1 null genotype (OR: 1.96, 95%CI=1.08-3.57), but no significant risk was found with the GSTP1 (Ile/Val) genotype (OR: 1.32, 95%CI = 0.74-2.37) and the GSTP1 Val/Val genotype (OR: 1.41, 95%CI=0.5-3.96) alone. Combined analysis of GSTM1 and GSTP1 showed significant higher risk associated with the GSTM1 (null/null) and GSTP1 [(Ile/Val)/ (Val/Val)] genotype (OR=2.78: 95%CI=1.16-6.69).

Key Words: Acute lymphoblastic leukemia - GSTP1 - GSTM1 - SNPs

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood, constituting about 30% of all childhood cancers (Chen et al., 1997). DNA damage in the hematopoietic precursor cell is an essential prerequisite for the development of leukemia and the normal cell has developed a series of mechanisms aimed at preventing such damage. One mechanism which can result in damage is mediated by reactive species generated either by environmentally encountered carcinogens or endogenously as a result of oxidative metabolism (Rollinson et al., 2002).

The glutathione-s-transferase (GST) super family of enzymes catalyzes the conjugation of xenobiotics and endogenous substances with Glutathione, and thereby plays a significant role in the inactivation, and occasionally the activation of many drugs and xenobiotics (Chen et al., 1997). Polymorphisms in glutathione-s-transferase (GST) enzymes produce significant alterations in the metabolism of many substrates, including carcinogens and chemotherapeutic agents (Orku et al., 2004). As a result, these polymorphisms have been suggested to play a role in the susceptibility to a variety of cancers like breast, lung, colorectal, in chemotherapy induced leukemia etc. (Lee et al., 2008; Ryberg et al., 1997; Ates et al., 2005; Allan et al., 2001).

GSTM1 is one of the genes encoding the mu class of enzymes located on 1p13.3, involved in the detoxification of polycyclic aromatic hydrocarbons and other mutagens, and cells from GSTM1 null individuals have been found to be more susceptible to DNA damage caused by these agents (Rossini et al., 2002). The frequency of the GSTM1 null genotype ranges from 23% to 48 % in African population; 39% to 62% in European population, 33% to 63% in Asian population (Cotton et al., 2000) and 22.4 % in South Indian population (Vettriselvi et al., 2006).

GSTP1 is a major enzyme involved in the inactivation of cigarette smoke carcinogens, such as benzo[a]pyrene diol epoxide, and other toxic constituents, such as acrolein. The GSTP1(Ile105Val) single nucleotide polymorphism which results from A313G transition in exon 5 of the GSTP1 gene replaces isoleucine at codon 105 with valine (I105V) within the active site of the enzyme and is associated with reduced enzymatic activity for certain substrates and altered thermostability (Sundberg et al.,1998; Xun-Hu et al., 1998; Ntais et al., 2005). The prevalence of the Val allele was 32% and 14% in control subjects of Caucasian and Asian descent, respectively (Ntais et al., 2005). Hydrophobic DNA adduct levels are found to be high in smokers with lung cancer carrying the GSTM1 (present/null) and GSTP1 (Ile105val) polymorphisms (Ryberg et al., 1997).

Studies have pointed to associations of GST polymorphisms with the increased risk of a variety of cancers in the Indian population including those of breast,

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cervix, oral cavity and prostate (Buch et al., 2002; Srivastava et al., 2005; Sharma et al.,2004; Samson et al., 2007). Our present study was aimed at examining the association of GSTM1 and GSTP1 polymorphisms in the susceptibility to acute lymphoblastic leukemia among South Indian population. This is the first study to our knowledge to report the frequency of GSTP1 polymorphisms in South Indian acute lymphoblastic leukemia patients.

Materials and Methods

Collection of samples:

The study group consisted of 92 immunophenotyped acute lymphoblastic leukemia patients (under 25 years of age) diagnosed at Cancer Institute (WIA), Chennai during the period 2004 to 2007. Inclusion criteria include 1) Morphologically and haematologically confirmed ALL 2) Age less than 25 years 3) Informed consent from the parent or patient (if >18 years of age). 8ml of peripheral blood/ 4ml of bone marrow were collected in EDTA at diagnosis. The control group comprised 150 cord blood samples collected in EDTA after obtaining informed consent from mothers delivering in the Andhra Mahila Sabha Hospital. All the samples were collected after obtaining ethical committee clearances from both the Institutions.

Genotyping:

DNA was extracted from lymphocytes using QIAmp DNA blood kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions. Quantitation of DNA was done using the Nanodrop spectrophotometer (ND 1000). The integrity of DNA was checked by PCR amplification of ABL gene. Genotyping for GSTM1 was done by the PCR method as described by Samson et al (2007) using exon-specific primers, (Forward 5'-CTGCCCTACTTGATTGATGGG-3'and Reverse 5'-CTGGATTGTAGCAGATCATGC-3'). The reaction mixture consisted of 50 ng of genomic DNA, 200 μM dNTPs, 10 pmoles each of forward and reverse primers, 10X buffer, 25 mM Mgcl2, and 0.5 units of AmpliTaq Gold polymerase (Applied Biosystems). The cycling conditions included an initial denaturation at 95˚C for 10 minutes, followed by 35 cycles of denaturation, annealing and extension at 95˚C, 64˚C and 72˚C for 1 minute each and a final extension at 72˚C for 5 minutes. The PCR products were run in 1.5% agarose gel containing Ethidium bromide. The presence of one or both the alleles was identified by a 273 bp fragment whereas its absence indicates the null genotype. Positive and negative controls were included in each run.

Genotyping of GSTP1 was done by Taqman Allelic discrimination assay using the ABI 7000 sequence detection system (Applied Biosystems, Foster City, CA).The primer and probe sequence was obtained from Cancer Genome Anatomy Project SNP500 Cancer Database.

Statistical analysis:

The association of GSTM1 (present/null) and GSTP1 (Ile105val) with acute lymphoblastic leukemia was been done by Chi-square test. Odds ratio (OR) were derived with 95% confidence interval. Further combined analysis of GSTM1 and GSTP1 was also done with GSTM1 (H/H) and GSTP1 (Ile/Ile) as the reference category.

Table 1. Frequency of GSTM1 (Present/Null) and GSTP1 (Ile105Val) Genotypes in ALL Patients (n=92) **and Controls** (n=150)

Variables	Cases	Controls	OR (95%CI)	P-value
GSTM1				
H/H	56	113	1.00*	
Null/Null	36	37	1.96 (1.08-3.57)	0.017
GSTP1				
Ile/Ile	43	81	1.00*	
Ile/Val	40	57	1.32 (0.74-2.37)	0.31
Val/Val	9	12	1.41 (0.5-3.96)	0.47

* Reference Category; GSTM1 (H/H) = GSTM1 (Homo/Hetero)

Results

The distribution of GSTM1 and GSTP1 polymorphisms among the acute lymphoblastic leukemia cases and controls are shown in Table 1. The GSTM1 null genotype was observed in 39 % of cases and 24.6 % of controls. We observed a statistically significant association of GSTM1 (null) with acute lymphoblastic leukaemia (p=0.017).

The GSTP1 allelic distributions among cases and controls were analysed. Among the cases, 46.7% were homozygous for wild type (Ile/Ile), 43.5% were heterozygous (Ile/Val) and 9.8% were homozygous for the variant (Val/Val). Among the controls, 54% were homozygous for the wild type (Ile/Ile), 38% were heterozygous (Ile/Val) and 8% were homozygous for the variant (Val/Val). We found that no significant risk was associated with GSTP1 (Ile/Val) to the development of ALL. The allelic frequencies of GSTP1 (Ile105Val) polymorphisms in cases and controls have been analysed (Ile=0.68, Val=0.31 in cases and Ile=0.73, Val=0.27 in controls) and the distribution of alleles were found to be in Hardy-Weinberg equilibrium.

Further combined effect of GSTM1 and GSTP1 was also analysed as shown in the Table 2, taking GSTM1 (null/null) and GSTP1 (Ile/Ile) as the reference category. We found a significant association of combined effect of GSTM1 (null/null) and GSTP1 [(Ile/Val)/ (Val/Val)] with the risk of ALL (p=0.011).

Table 2. Combined analysis of GSTM1 (Present/Null) and GSTP1 (Ile105Val) Genotypes and the Relative Risk of ALL

Variables	Cases	Controls	OR (95%CI)	P-value
GSTM1 (H/H)/ GSTP1(Ile/Ile)	27	60	1.00*	
GSTM1(H/H)/ GSTP1{(Ile/Val)/(V/V)}	29	53	1.22 (0.61-2.43)	0.55
GSTM1 (-/-)/ GSTP1(Ile/Ile)	16	21	1.69 (0.71-4.03)	0.19
GSTM1(-/-)/ GSTP1{(Ile/Val)/(V/V)}	20	16	2.78 (1.16-6.69)	0.011

* Reference Category; GSTM1 (H/H) = GSTM1 (Homo/Hetero)

Discussion

ALL is a heterogenous disease characterised by the predominance of lymphoblasts or immature hematopoietic precursors, in which malignant cells express diverse phenotypes and respond very variably to different chemotherapeutic regimens (Krajinovic et al., 1999). GSTs play a critical role by protecting against the reactive oxygen species caused by breakdown of peroxidized lipids and capable of oxidising DNA and generating damage (Rollinson et al. 2002).

In the present study, we have used cord blood samples as controls instead of age matched controls due to ethical issues and difficulty in obtaining blood sample with informed consent. Wiemels et al (2001) and Alcasabas et al (2008) have used cord blood samples as controls to check MTHFR polymorphisms in the ALL patients. The cord blood samples in the present study were consecutive samples collected during a period of three months. It was estimated that six thousand children among 882 million population in India develop ALL each year (Chandy, 1995). The probability of one child among 150 healthy newborns to develop ALL compared to the incidence rate is negligible.

Previous reports on the association of the GSTM1 (Present/null) to the susceptibility of acute lymphoblastic leukemia were contradictory. Some studies showed significant association of GSTM1 (Null/null) genotype to the susceptibility to ALL (Krajinovic et al.,1999; Alves et al., 2002; Pakakasama et al., 2005) whereas, other studies revealed a lack of any significant association (Davies et al., 2002; Rollinson et al., 2002; Haranatha et al.,2006). In our study, we have shown that the GSTM1 (null/null) genotype significantly increases the risk of developing acute lymphoblastic leukemia nearly two fold (OR= 1.96: 95% CI= 1.08-3.57). This is in concordance with the results of Joseph et al (2004), who used age-matched controls.

Our study showed that GSTP1 (Ile105Val) by itself is not associated with the risk of developing ALL. This is in concordance with the results of Gatedee et al (2007) and Rollinson et al (2002) but differs from Renata et al (2004) and Zubowska et al (2004) who reported an increased risk of ALL associated with GSTP1 polymorphisms. We have analysed the combined effect of GSTM1(Present/Null) and GSTP1(Ile105Val) taking GSTM1 (H/H) and GSTP1 (Ile/Ile) as the reference category. We found a significant risk associated with the combination of GSTM1 (null/null) and GSTP1 [(Ile/Val)/(Val/Val)] to the predisposition of ALL (OR=2.78; 95% CI=(1.16-6.69). Though GSTP1 (Ile/Val) alone was here not found to be a risk factor, when it is combined with GSTM1 (null/null), it appears that it might increase the risk of developing ALL.

In conclusion, our study has shown a statistically significant increased risk associated with GSTM1 (null/null) genotype in the predisposition of acute lymphoblastic leukemia. Though GSTP1 (Ile105val) SNP alone did not show any increased risk, when it is combined with GSTM1 (null/null) genotype, a greater increase in risk of ALL has been observed.

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