

RESEARCH COMMUNICATION

Polymorphic Variation in Glutathione-S-transferase Genes and Risk of Chronic Myeloid Leukaemia in the Kashmiri Population

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Abstract

Cancer is a complex disease and the genetic susceptibility to it could be an outcome of the inherited difference in the capacity of xenobiotic metabolizing enzymes. Glutathione-S-transferases (GSTs) are phase II metabolizing enzymes whose various genotypes have been associated with increased risk of different types of cancer. Null mutations caused by the deletion of the entire gene result in the absence of the enzymatic activity and increase in the risk of developing cancer including chronic myeloid leukaemia (CML). In the present case-control study we evaluated the effect of null mutations in GSTM1 and GSTT1 genes on the risk of developing CML. The study included 75 CML patients (43 males and 32 females; age (mean \pm S.D) 42.3 \pm 13.4 years) and unrelated non-malignant controls (76 male and 48 females; age (mean \pm S.D) 41.5 \pm 12.9). The distribution of GSTM1 and GSTT1 genotypes in CML patients and controls was assessed by multiplex-PCR method. Logistic regression was used to assess the relationship between GSTM1 and GSTT1 genotypes and risk of CML. Chi-square test was used to evaluate the trend in modulating the risk to CML by one or more potential high risk genotype. Although GSTM1 null genotype frequency was higher in CML patients (41%) than in the controls (35%), it did not reach a statistical significance (OD = 1.32, 95% CI: 0.73–2.40; P value = 0.4295). The frequency of GSTT1 null genotypes was higher in the CML patients (36%) than in the controls (21%) and the difference was found to be statistically significant (OD = 2.12, 95% CI: 1.12–4.02; P value = 0.0308). This suggests that the presence of GSTT1 genotype may have protective role against the CML. We found a statistically significant (OD = 3.09, 95% CI: 1.122–8.528; P value = 0.0472) interaction between the GSTM1 and GSTT1 null genotypes and thus individuals carrying null genotypes of both GSTM1 and GSTT1 genes are at elevated risk of CML.

Keywords: Glutathione S transferase - CML - GSTM1 - GSTT1 - genetic polymorphisms

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Introduction

Chronic myeloid leukemia or chronic myelocytic leukemia (Ghanei and Vosoghi, 2002) is a type of blood or bone marrow cancer which is characterized by an abnormal increase of white blood cells, by the presence of the Philadelphia chromosome and the t(9;22)(q34;q11) translocation (Rowley, 1973; Faderl et al., 1999). CML is more prevalent in males as compared to females (Redaelli et al., 2004). A number of factors have been implicated to play role in the development of this disease. Several studies have shown the association between genetic alteration/s in the precursor hematopoietic cells with the risk to develop CML (Meggyesi et al., 2011). Exposure to endogenous or exogenous toxic substances can lead to genetic alterations and hence increased susceptibility to cancer. Cells have developed an effective mechanism to prevent accumulation of damaging xenobiotics by way of their elimination catalyzed by multiple enzyme

system. The enzymes of the multiple enzyme system are classified in two categories namely Phase I and Phase II. Phase I enzymes like Cytochrome P450 can activate the carcinogens directly and produce active metabolites while phase II enzymes like Glutathione-S-transferase can detoxify and process the activated metabolites for final breakdown. Hence the toxicological outcome of exposure, absorption and activation/detoxification of xenobiotics depends on a delicate balance between the phase I and phase II enzymes. The activation of the precarcinogens to carcinogens by way of hydrolysis, oxidation or reduction leads to the formation of various reactive species which produce DNA adducts which in turn leads to mutations in oncogenes or tumor suppressor genes and hence carcinogenesis. Inter-individual differences in the ability to activate pro-carcinogens or detoxify potential carcinogen may account for large differences in the susceptibility to cancer. Thus the individuals who have reduced ability to detoxify toxic substances from the

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body are at an increased risk to develop cancer. Further an association has been reported between the polymorphic forms of the xenobiotic metabolizing enzymes and the altered risk to various cancers including the CML (Hishida et al., 2005).

GSTs are a family of dimeric biotransformation enzymes. These Phase II enzymes catalyze the binding of a large variety of reactive electrophiles to the sulfhydryl group of glutathione, changing them into more hydrophilic excretable form and thus play a crucial role in the detoxification of toxic substances. GSTs are comprised of the four main classes; alpha (α) (GSTA), mu (μ) (GSTM), pi (π) (GSTP) and theta (Θ) (GSTT) (Hayes and Pulford, 1995). Several studies have reported the association of the polymorphic forms of the GSTs especially GSTTM1 and GSTT1 null genotypes with the various types of cancer including CML (Garte et al., 2000; Quiñones et al., 2001; Cai et al., 2001; Aktas et al., 2004; Canalle et al., 2004;). The chief polymorphic form of the GSTM1 is represented by a non-functional null allele (McLellan et al., 1997), which is proposed to result due to an unequal crossing over between two highly identical 4.2Kb deletions that includes the entire GSTM1 gene (Xu et al., 1998). The polymorphic form of GSTT1 results in a partial or complete deletion of the gene, which has been found to cause deficiency in enzyme activity (Hallier et al., 1993). Varied results have been reported on the GSTM1 and GSTT1 null genotype and its association to CML in different ethnic populations (Weber, 1999; Roy et al., 2001; Hishida et al., 2005; Mondal et al., 2005; Lourenco et al., 2005).

Till date no study has been done to understand the aetiology of CML in Kashmir. The main aim of the present study was to analyze the influence of the polymorphism of GSTM1 and GSTT1 genes on the susceptibility of CML in Kashmiri population.

Materials and Methods

Subject recruitment

This hospital based case-control study was conducted following approval by the ethical committee of Sher-i-Kashmir Institute of Medical Science (SKIMS), India and the subjects (CML patients as well as controls) were included only after they willingly decided to become

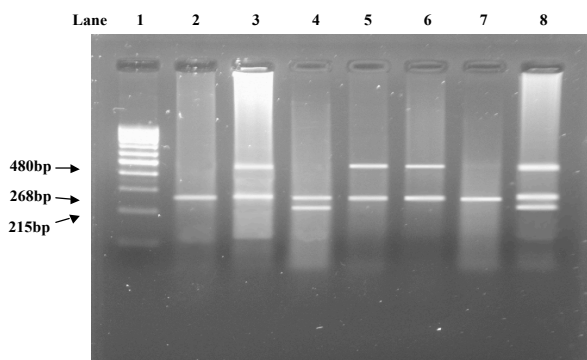


Figure 1. 2% Agarose Gel Demonstrating Multiplex PCR Genotyping of Genomic DNA Samples for Detection of GSTM1 and GSTT1 Null Genotypes

the part of the study. The study was conducted over the period of fourteen months starting from May 2010 upto September 2011. The inclusion of CML patients was based on the proper diagnosis which included standard clinico-hematological criteria and the presence of BCR-ABL fusion gene. Gender, age (± 7 years) and geography matched subjects who did not have had any malignancy were included as controls in the study. A total of 75 CML cases (males 43 (57.3%) and females 32 (42.6%) with a mean age of 42.3 ± 13.4) were recruited from the Department of Clinical Hematology, SKIMS and during the same period 124 volunteer controls (males 76 (61.2%) and females 48 (38.7%) with the mean age of 41.5 ± 12.9) were also recruited from various OPDs of the same hospital. Five ml of venous blood was collected after taking consent from each subject in a sterile EDTA coated vials and was stored at -80°C . Genomic DNA was isolated from the blood samples by using Phenol-Chloroform method (Sambrook, 2001) and the isolated DNA was stored at -20°C for future use.

GSTM1 and GSTT1 polymorphism

The polymorphism of GSTM1 and GSTT1 was assessed by multiplex-PCR reaction (Arand et al., 1996) using β -globin as the positive control. The forward and reverse primers used for GSTM1 gene were 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 5'-GTT GGG CTC AAA TAT ACG GTG G-3' respectively, which on amplification produced a 215-bp product, and for GSTT1, the forward and reverse primers used were 5'-TTC CTT ACT GGT CCT CACATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3' respectively, which on amplification produced a 480-bp product. β -globin gene on amplification produced a 268bp product and its absence indicated the failure of PCR.

PCR was performed in a $25\mu\text{l}$ reaction containing 50 ng of genomic DNA, $200\mu\text{M}$ dNTPs, 10X PCR buffer of $1.5\mu\text{M}$ MgCl_2 , 1U Taq polymerase (Fermentas) and 20 pmol of each pairs of primer. Initial denaturation at 94°C for 5 minutes was followed by 35 cycles of 1 minute at 94°C , 1 minute at 57°C , 1 minute at 72°C and final extension for 7 minutes at 72°C . The PCR products were analyzed on 2% agarose gel containing ethidium bromide. The absence of 215bp product for GSTM1 and 480bp product for GSTT1 in the presence of 268bp product of β -globin gene indicates their null genotype.

Statistical analysis

Odds ratio (OR) with 95% confidence limits calculated by logistic regression was used to assess the relationship between GSTM1 and GSTT1 genotypes and risk of CML. Chi-square test was used to evaluate the trend in modulating the risk to CML by one or more potential high risk genotype. A probability value (P-value) of <0.005 was taken statistically significant. All the statistical calculations were done using SPSS 15.0.

Results

The study was conducted over a period of eighteen months starting from May 2010 to September 2011. This

Table 1. The Distribution of GSTM1 and GSTT1 Genotypes in CML Patients and Controls

Poly-morphism	Cases n (%)	Control n (%)	OR (95% CI)	P value
GSTM1				
Present	44 (59%)	81 (65%)	1	-
Null	31 (41%)	43 (35%)	1.32 (0.73 - 2.40)	0.4295
GSTT1				
Present	48 (64%)	98 (79%)	1	-
Null	27 (36%)	26 (21%)	2.12 (1.12 - 4.02)	0.0308

Table 2. Combined Effect of GSTM1 and GSTT1 Null Genotypes on CML risk

GSTM1	GSTT1	Cases	Controls	OR	95% CI	P value
Present	Present	28	63	-	-	-
Present	Null	16	18	2.00	0.954-2.450	0.1372
Null	Present	20	35	1.28	0.633-2.608	0.6062
Null	Null	11	8	3.09	1.122-8.528	0.0472

case-control study included well-confirmed 75 patients with CML and 124 unrelated non-malignant controls. The distribution of genotypic frequencies of GSTM1 and GSTT1 in controls and patients suffering from CML is summarised in Table 1.

The frequency of the GSTM1 (Present) and GSTM1 null genotypes in the controls were 65% (81/124) and 35% (43/124), respectively. Although the frequency of GSTM1 null genotype was higher in CML patients than in the controls but it was not statistically significant. The frequency of GSTT1 present, individuals carrying GSTT1 gene and GSTT1 null genotypes (individuals lacking the GSTT1 gene) in CML patients were 64% (48/75) and 36% (27/75), respectively. The frequency of the GSTT1 (Present) and GSTT1 null genotypes in the controls were 79% (98/124) and 21% (26/124), respectively. It was found that the frequency of GSTT1 null genotypes was higher in the CML patients than in the controls. This indicates the presence of GSTT1 genotype may have protective role against the CML. The combined effect of GSTM1 and GSTT1 null genotypes on CML risk is given in the Table 2. We found a statistically significant interaction between the GSTM1 and GSTT1 null genotypes and thus the individuals carrying null genotype of both GSTM1 and GSTT1 genes are at a higher risk to CML.

Representative 2% agarose gel picture showing multiplex PCR genotyping of genomic DNA samples for detection of GSTM1 and GSTT1 null genotypes is given in the Figure 1. The absence of 215 bp band indicates GSTM1 null genotype; absence of 480 bp band indicates GSTT1 null genotype; β -globin was used as internal positive control and was co-amplified in all samples. Lane 1 - Marker 100bp DNA ladder, Lane 4- GSTT1 null genotype. Lane 3, 5 and 6- GSTM1 null genotype. Lane 8- GSTM1 & GSTT1 positive genotype. Lane 2, 7- GSTM1 & GSTT1 null genotype.

Discussion

CML is a myeloproliferative disorder but definite mechanism leading to this carcinogenesis is yet to be understood completely. The known genetic abnormality

associated with the CML is the condition known as Philadelphia chromosome, which occurs as a result of reciprocal translocation between chromosome 9 and 22 leading to juxtaposition of BCR-ABL gene. This condition leads to increased tyrosine kinase activity which in turn initiates and maintains the leukemic condition (Deininger, 2000). Only a limited number of studies have been so far conducted in which the role of other possible confounding factors like environment has been studied together with genetic analysis. Only causative factor known to be associated with CML is exposure to radioactivity. Individual genotypic differences and also the level of expression of these carcinogen-metabolizing enzymes are crucial in determining the susceptibility of developing the cancer (Kawajiri et al., 1993). The GSTM1 and GSTT1 enzymes function in the biotransformation of various toxic substances. Polymorphic forms of the GSTs (GSTM1 and GSTT1) have been reported and the variation in genotype from the wild form may have relevance in determining the susceptibility to cancer. Individuals with less efficient phase II metabolising enzymes have been found to be at a greater risk to develop cancer (Astrup, 2000). Null mutations of GSTM1 and GSTT1 genes have been linked with an increase in a number of cancers, likely due to an increased susceptibility to environmental toxins and carcinogens (Tan et al., 2000). In the present study we analysed the GSTM1 and GSTT1 null mutations in CML cases and controls as these two null mutations separately or in combination have been reported to modulate the risk of CML. Variable frequencies of xenobiotic metabolizing polymorphic alleles in different populations reflect the difference in susceptibility to certain cancers. Loureno et al, 2005 reported that in Brazil the individuals with GSTM1 (OR = 0.98; 95% CI: 0.65–1.50), GSTT1 (OR = 1.06; 95% CI: 0.62–1.80) and combined null genotypes (OR = 0.84; 95% CI: 0.36–1.96) are at similar risk of CML as compared with those without the null genotypes (Rollinson et al., 2005). Similar results were also reported by Chen et al., 2008, where no significant difference was observed in the frequencies of GSTM1, GSTT1 or combined genotypes between cases and controls (Chen et al., 2008).

In the present study, we found that although the GSTM1 null genotype frequency was higher in CML patients (41%) than in the controls (35%), however it did not reach a statistical significance (OR = 1.327, 95% CI: 0.73–2.40; P value = 0.4295) (Table 1). These findings are in agreement with the study conducted by Bajpai et al. (2007) in which they did not find any significant increase in the risk to CML due to GSTM1 null genotype (OR = 1.30, 95% CI = 0.65–2.63, P = 0.5302) (Bajpai et al., 2007). Similar results were also reported by Manzoor et al., (2010) for GSTM1 gene polymorphism in esophageal cancer in Kashmir (OR = 1.50, 95% CI) = 0.96–2.35, P = 0.075) (Malik et al., 2010).

We also evaluated the potential role of GSTT1 polymorphism and CML risk. Our results suggest a positive association between the GSTT1 null mutation and the risk to develop CML. The frequency of GSTT1 null genotypes was higher in the CML patients (36%) than in the controls (21%) and the difference was found to be

statistically significant (OD = 2.12, 95% CI: 1.12–4.02; P value = 0.0308) (Table 1). Therefore, it might be thought that the wild type of the GSTT1 (i.e., when GSTT1 gene is present) is a protective factor against CML. These findings are in agreement with the study conducted by Bajpai et al., (2007) in which they found a significant increase in the risk to CML due to GSTT1 null genotype (OR= 2.67, 95% CI: 1.03–7.01, P = 0.0417) (Bajpai et al., 2007). Also in previous studies done by Rollinson et al, (2000), GSTT1 null genotypes were shown to be risk factors for both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (Rollinson et al., 2000).

Interaction between the GSTM1 and GSTT1 gene was assessed by calculating the trend in risk of CML associated with any potential high risk genotypes (Table 2). We found a statistically significant (OD = 3.09, 95% CI: 1.122–8.528; P value = 0.0472) interaction between the GSTM1 and GSTT1 null genotypes and thus the individuals carrying null genotype of both GSTM1 and GSTT1 genes are at a higher risk to CML. It might be inferred from the data that the two genes (GSTM1 and GSTT1) act in a synergistic way and that they are an important part of the detoxification system.

This is the first report highlighting the genetic susceptibility due to polymorphisms in GSTM1 and GSTT1 genes in the Kashmiri population and the risk to CML. The strength of this study is that it contributes significantly to the understanding of the role of polymorphic variants of various phase II metabolizing genes in modulating the individual susceptibility to CML in the Kashmir valley.

In conclusion, our study reveals that the GSTT1 null genotype separately and also in combination with the GSTM1 null genotype increases the risk of CML in Kashmiri population. However, this should be considered as only a preliminary result and requires further substantiation with larger sample size.

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