RESEARCH COMMUNICATION

Gluthatione-S-transferase T1-null Genotype Predisposes Adults to Acute Promyelocytic Leukemia; a Case-Control Study

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Abstract

Polymorphisms of glutathione S-transferase (GST) proteins are correlated with elevated risk of many cancers including hematologic malignancies. Particularly concerning acute promyelocytic leukemia (APL), the studies on association between GSTM1, GSTT1 and GSTP1 and the disease predisposition are scarce and contradictory. The aim of this study was to examine whether polymorphic variations in GST confer susceptibility to APL. GSTM1 and GSTT1 null and GSTP1 Ile105Val alleles were determined using polymerase chain reaction (PCR) and PCR-RFLP, respectively, in 114 APL patients and 99 healthy controls. Frequency of GSTT1 null and GSTM1 null genotypes were higher in APL group which it was statistically significant for GSTT1 null ($p< 0.01$). The GSTM1 null and GSTT1 null conferred a 1.36-fold (OR= 1.36, 95% CI = 0.79-2.33, p= 0.18) and 2.14-fold (OR= 2.14; 95% CI: 1.18-3.92, p= 0.013) increase in risk of APL, respectively, relative to the presence of the GSTM1 or GSTT1 genes. GSTP1 Ile105/Val105 and Val105/Val105 genotypes showed no increase in the risk of APL (OR= 0.94; 95% CI: 0.52-1.67 and OR= 1.12; 95% CI: 0.48-2.60, respectively). Our results suggest that GSTT1 null genotype may be associated with increased risk of APL.

Keywords: GSTM1 - GSTT1 - GSTP1 - polymorphism - acute promyelocytic leukemia - Iran

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Introduction

Acute leukemia, including acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), is a malignancy which is seen in any ages. AML consists 45% of all blood malignancies. Acute promyelocytic leukemia (APL) or FAB AML-M3 is a subtype of myeloid malignancies which accounts for about 10-15% of AML. APL is characterized by reciprocal chromosomal translocation, t(15;17), which results in PML/RARα fusion protein production and subsequent promyelocyte differentiation block (Grignani et al., 1994). Despite widespread studies about the mechanisms of leukemia, the exact causes are not known. Similar to other cancers, APL is considered as a complex disease which is affected by combination of environmental and genetically factors and there have been increasing evidences about the role of genetic susceptibility to development of acute leukemia. It is believed that DNA damage by the reactive oxygen species in the hematopoietic precursor cells is an important factor in the development of leukemia.

Gluthatione-S-transferase (GST) is a group of polymorphic enzymes of phase II which catalyse conjugation of glutathione onto the reactive electrophillic agents on the exogenous and endogenous compounds. Three more important polymorphisms of GSTs are M1 (GSTM1 null allele) and T1 (GSTT1 null allele) genes deletions and one transition point mutation of A1578>G that creates the GSTP1 Ile105Val polymorphism with reduced activity (Mannervik et al., 1985; Ali-Osman et al., 1997; Rebbeck. 1997). Accumulating body of evidence points toward an important role of GST polymorphisms in aetiology of some disease and some malignancies including acute leukemias (Rebbeck. 1997; Hayes and Strange. 2000; Ye and Song. 2005). In the past few years, several studies associating the presence of the GSTM1 and GSTT1 polymorphisms with acute myeloid leukemias have been performed (Lemos et al., 1999; Sasai et al., 1999; Crump et al., 2000; Davies et al., 2000; Naoe et al., 2000; Allan et al., 2001; Arruda et al., 2001; Haase et al., 2002; Souza et al., 2008). However, particularly concerning APL, few studies have been conducted and have reported contradictory results for association between GSTM1 and GSTT1 and APL predisposition, without considering the role of GSTP1. For instance, Crump et al. (2000) analyzing a case-control study, reported no significant association between the deletion of GSTM1 and GSTT1 and increased risk of AML-M3. Recently Souza et al. (2008) have studied the susceptibility role of polymorphisms of GSTM1 and GSTT1 to AML including

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23 APL patients. They have found only GSTT1 null genotype was significantly more frequent among APL patients. Rollinson et al. (Rollinson et al., 2000) showed a 3.22-fold and 1.54-fold increases in the frequency of GSTT1 and GSTM1 deletions in 55 APL patients, respectively. Furthermore, to evaluate the relation of GST polymorphisms and susceptibility to chemotherapy induced leukemia including APL, Allan et al. (Allan, Wild et al., 2001) have found the significant role of GSTP1 but unfortunately they did not stratify the patients according to their FAB subgroups. To clarify an association between genotypes and cancer risks, sample size is considered to be a crucial factor in the design of case-control studies(Ye and Song. 2005), and thus far, the relationship between GST genotypes and APL risk has not been studied in a large numbers of subjects. Here we established this study with a large sample population to test the distribution of GSTM1, GSTT1 and GSTP1 polymorphisms among APL patients.

Materials and Methods

Patients enrolled in the study were 114 adult patients with acute promyelocytic leukemia, 47% male and 53% female who were selected between 2000 and 2007 from Hematology, Oncology and BMT Research Center (HOBMTRC) of Shariati Hospital, Tehran, Iran. The diagnosis of APL was established according to the clinical presentation and morphological criteria of the French-American-British (FAB) classification and was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for PML/RARα transcripts as described before (Ghavamzadeh et al., 2006; Mandegary et al., 2010). The control group consisted of 100 (44% male and 56% female) healthy bone marrow donors.

DNA Extraction and GST genotyping

Genomic DNA was isolated from EDTA whole blood using a rapid salting out DNA extraction method (Miller et al., 1988). The DNA yield was estimated by measuring the optical density at 260 nm in a spectrophotometer prior to PCR reaction and then aliquots of DNA were stored in Tris-EDTA buffer at -70 °C until analysis of GST genotypes. A multiplex PCR was performed to detect the presence or absence of the GSTM1 and GSTT1 genes, according to the protocol described in our previous article with this exception that as internal positive control, c-Abl gene was used instead of β-globin (Moradi et al., 2009). This technique conclusively identified GSTM1 null and GSTT1 null genotypes, corresponding to the deletion of both alleles.

Primers to amplify the GSTM1(X68676.1, GeneBank) genotypes were 5'-GAA CTC CCT GAA AAG CTA AAG C-3' (2440-2461 bp) and 5'-GTT GGG CTC AAA TAT ACG GTG G-3'(2637-2658 bp) as forward and reverse primers, respectively, resulting in a 219 bp band, and for the GSTT1 (AB057594.1, GeneBank) were 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' (9350-9372 bp) and 5'-TCA CCG GAT CAT GGC CAG CA-3' (9780-9808 bp) as forward and reverse primers, respectively, resulting in a 450 bp band. As an internal control, the c-Abl gene was amplified using 5-TTC AGC GGC CAG TAG CAT CTG ACT-3' and 5'-TGT GAT TAT AGC CTA AGA CCC GGA GCT TTT-3' as forward and reverse primers, respectively, giving a 750 bp product. PCR reactions were resolved in an ethidium bromide-stained 2% agarose gel electrophoresis. The absence of the GST M1- or GST T1-specific fragments indicated the corresponding null genotype, whereas the c-Abl specific fragment confirmed the presence of amplifiable DNA in the reaction mixture. For GSTP1 (M24485.1, GeneBank) genotyping, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used. The primers were: forward- 5'-CCA ACC CCA GGG CTC TAT G-3' (2537-2555 bp) and reverse-5'-GCA GGT TGT GTC TTG TCC CAG-3' (2805-2825 bp). The 288-bp PCR product was subsequently digested by the restriction enzyme Alw26I (Fermentase, Lithuania) for 6 h at 37°C. The samples were then analyzed by electrophoresis in 3% agarose gels and visualized by ethidium bromide staining. The homozygous Ile genotype was identified by a 280 bp band, the homozygous Val genotype was identified by the presence of 150 and 124 bp bands. The heterozygous type exhibited all three bands.

PCR reaction mixture contained 2 mM of MgCl2, 0.2 mM of dNTP, 1x PCR buffer, 1.2 U of Taq DNA polymerase, 100 ng of DNA template and 5 pmol/ml of both forward and reverse primers in deionized sterile water in total volume of 50 ml. Amplification was carried out with touch-down method as follows: 94 °C for 30 s (denaturation), 60 °C (-0.5 °C/ cycle, 10 cycle) for 30 s (annealing) and 72 °C for 30 s (extension), then 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s for 35 cycle and 72 °C for 10 min as final extension.

Statistical Methods

The principal measure of interest was the relative risk of APL for individuals with the GSTT1 and GSTM1 null and GSTP1 Val/Val genotypes compared with individuals without the GSTM1 and GSTT1 null genotype and GSTP1 Ile/Ile. Statistical significance for odd ratio was calculated using Chi-square test. Odds ratio (OR) was calculated separately with 95% confidence interval (95% CI) for genotype frequencies in APL patients as compared with respective controls. Sample sizes were planned so as to have 80% power for detecting an OR of 1.5 for the association between the GSTT1 gene deletion and APL. All analyses were conducted using Statistics Package for

Table 1. Impact of Single Genotypes of GST on the Frequency of APL

Genotype	Controls	APL	OR (95% CI)	p-value
GST-M1	(n=99)	(n=114)		
Present	51 (51.5%)	50 (43.8%)	1	
Null	48 (48.5%)	64 (56.2%)	1.36(0.79-2.33)	NS
GST-T1	(n=99)	(n=114)		
Present	76 (76.7%)	69 (60.5%)	1	
Null	23 (33.3%)	45 (39.5%)	2.14(1.18-3.92)	0.013
GST-P1	(n=105)	(n=110)		
Ile-Ile	54 (51.4%)	56 (50.9%)	1	
Ile-Val	37 (35.2%)	41 (37.3%)	0.94 (0.52-1.67)	NS
Val-Val	14 (13.3%)	13 (11.8%)	1.12(0.48-2.60)	NS

Table 2. GST Combination Genotypes and Odds-Ratios for Risk of APL

GST M1	GST T1	GST P1	APL (N= 113)	Controls (N= 99)	OR	95% CI	p-Value
present	present	-	37 (32.7)	39 (39.4)	1		
present	null	-	13 (11.5)	12 (12.1)	1.14	0.46-2.82	2.82
null	present	-	32 (28.3)	37 (37.4)	0.91	0.47-1.75	0.45
null	null	-	32 (28.3)	11 (11.1)	3.10	1.35-6.96	0.007
present	present	Ile/Ile	19 (16.8)	24 (24.3)	1		
present	present	Ile/Val + Val/Val	17 (15.0)	12 (12.1)	1.78	0.69-4.65	0.17
present	null	Ile/Ile	10 (8.8)	5 (5.1)	2.52	0.74-8.62	0.12
present	null	Ile/Val + Val/Val	4 (3.5)	7 (7.1)	0.72	0.18-2.83	0.45
null	present	Ile/Ile	11 (9.7)	16 (16.2)	0.87	0.33-2.30	0.49
null	present	Ile/Val + Val/Val	20 (17.7)	21 (21.2)	1.20	0.51-2.84	0.4
null	null	Ile/Ile	12 (10.6)	8 (8.1)	1.88	0.64-5.55	0.18
null	null	Ile/Val + Val/Val	20 (17.7)	4 (4.0)	6.25	1.85-21.73	0.002

Social Sciences (SPSS Inc., USA) software for Windows.

Results

DNA of 114 patients with RT-PCR diagnosed APL and 99 healthy controls of a similar ethnic background were analyzed for genotypes of GSTM1, GSTT1 and GSTP1. Some individuals were not successfully genotyped for all genotypes tested, thus explaining the variations in the total number of samples listed in tables. The frequency of GSTM1, GSTT1, GSTP1genotypes in APL patients and in controls are given in Table 1. The observed prevalence of these genotypes is in accordance with data from other studies of groups of Iranians (Saadat and Saadat. 2001; Mohammadzadeh Ghobadloo et al., 2006; Alidoust et al., 2007; Torkaman-Boutorabi et al., 2007).

Allele and genotype frequencies of GST are presented in Table 1. The results showed a higher but not statistically significant frequency of GSTM1 gene deletion in the APL group when compared with the control group [64 versus 48%, P= 0.18, OR= 1.36 (95% CI 0.79–2.33)]. The incidence of GSTT1 null genotype was significantly (p= 0.008) increased in APL cases as compared with controls [45 versus 23%, P< 0.01, OR= 2.14 (95% CI 1.18–3.92)] (Table 1). Frequencies of GSTP1Ile/Val and Val/Val genotypes in the control group were 37 and 14%, respectively. The frequency of Ile/Val and Val/Val genotypes in the APL group showed no increase (Table 1) [41 versus 13% and 13 versus 14%, P > 0.05, odds ratios 0.94 (95% CI 0.52–1.67) and 1.12 (95% CI 0.48-2.60), respectively].

In order to assess the combined effects of polymorphic status at the GST T1, GST M1 and GST P1 loci on the risk of APL, any interaction between the 3 GST genotypes was calculated (Table 2). Individuals carrying nondeleted GSTM1 and GSTT1 and GSTP1-Ile105Ile served as the reference group. Heterozygotes and homozygotes for the GSTP1-105Val allele were combined for the analysis.

Increased risk estimates were observed when both GSTT1 and M1 alleles were null. The frequency of individuals presenting with a combination of the homozygous deletion of GST T1 and GST M1 are shown to be significantly increased in the APL group compared with controls [32 versus 11%, P< 0.01, OR= 3.1 (95% CI 1.35–6.96)]. For APL the greatest estimated risk was for GST T1 null, GST M1 null and GST P1 Val/Val (OR= 6.25, 95% CI 1.85-21.73).

Discussion

The relationship between GST polymorphisms and risk of a variety of common cancers including hematologic malignancies such as acute lymphoblastic and myeloid leukemia has been reported (Lemos et al., 1999; Crump, Chen et al., 2000; Saadat and Saadat. 2000; Arruda et al., 2001; Alves et al., 2002; Ye and Song. 2005; Souza et al., 2008). However, particularly concerning APL, few studies on the AML including small APL cases until now have been performed to study the association of GSTM1 and GSTT1 polymorphisms and APL, without considering the role of GSTP1 (Crump et al., 2000; Davies et al., 2000; Rollinson et al., 2000; Allan et al., 2001; Souza et al., 2008).

Previous reports on GST null genotypes as a risk factor for APL gave heterogeneous results. A study from Crump et al. (2000) reported no association between GSTT1 null and GSTM1 null genotypes and risk of APL (OR= 0.94; 95% CI: 0.34–2.57 and 0.80; 95% CI: 0.37–1.69, respectively). A recent study from the Brazil found an statistically significant increased risk for APL in adults with the GSTT1 null genotype (OR= 2.75; 95% CI: 1.10-6.88, p< 0.04) and combination of GST null genotypes (OR= 3.61; 95% CI: 1.37-9.51, p< 0.05) (Rollinson et al., 2000). Arruda et al. (2001) found an association between the development AML and the GSTM1 null or GSTT1 null genotypes among Brazilian individuals, but they did not separate the FAB subgroups. Rollinson et al. (2000) analyzed the association between the GSTM1 or GSTT1 deletions and increased risk to AML. Analyzing the data according to the FAB types of AML, they found significant increase in GSTT1 null genotype in APL patients comparing to their control cases (OR= 3.22; 95% CI: 1.09-9.49).

In our present study we found that carrying GSTT1 null genotypes significantly increases the risk of developing APL, the risk becoming higher with possession of both GSTT1 null and GSTM1 null and the risk being highest with possession of all 3 GSTT1 null, GSTM1 null and GSTP1 hetero/homozygote Val genotypes. In conclusion, our current findings propvide strong evidence that GSTT1 null genotype and its combination with GSTM1 null and GSTP1 Val hetero/homozygote may increase the risk of

adult APL.

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