RESEARCH ARTICLE

Deletion of GSTM1 and T1 Genes as a Risk Factor for Development of Acute Leukemia

Nageswara Rao Dunna[1], Sugunakar Vure[2], K Sailaja[2], D Surekha[2], D Raghunadharao[3], Senthil Rajappa[3], S Vishnupriya[2]*

Abstract

The glutathione S-transferases (GSTs) are a family of enzymes involved in the detoxification of a wide range of chemicals, including important environmental carcinogens, as well as chemotherapeutic agents. In the present study 294 acute leukemia cases, comprising 152 of acute lymphocytic leukemia (ALL) and 142 of acute myeloid leukemia, and 251 control samples were analyzed for GSTM1 and GSTT1 polymorphisms through multiplex PCR methods. Significantly increased frequencies of GSTM1 null genotype (M0), GSTT1 null genotype (T0) and *GST* double null genotype (T0M0) were observed in the both ALL and AML cases as compared to controls. When data were analyzed with respect to clinical variables, increased mean levels of WBC, Blast %, LDH and significant reduction in DFS were observed in both ALL and AML cases with T0 genotype. In conclusion, absence of both *GST M & GST T* might confer increased risk of developing ALL or AML. The absence of *GST* enzyme might lead to oxidative stress and subsequent DNA damage resulting in genomic instability, a hallmark of acute leukemia. The GST enzyme deficiency might also exert impact on clinical prognosis leading to poorer DFS. Hence *GST* genotyping can be made mandatory in management of acute leukemia so that more aggressive therapy such as allogenic stem cell transplantation may be planned in the case of patients with a null genotype.

Keywords: Glutathione S- transferase - null phenotype - acute leukemia - risk factor - prognostic factor

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Introduction

Glutathione S transferases (*GSTs*), super family of dimeric phase II metabolizing enzymes, play an important role in the cellular defense system. GST enzymes catalyze the conjugation of toxic and carcinogenic electrophilic molecules with glutathione and thereby protect cellular macromolecules from damage (Boyer et al., 1985). Thus GST enzymes regulate cytotoxicity of a variety of chemotherapeutic drugs (Hoban et al., 1992). Glutathione S-transferases (*GSTs*) constitute a family of enzymes encoded by five gene families μ, θ, π, α, σ which are involved in phase II metabolism and implicated in the detoxification of a broad range of compounds, including xenobiotics, pesticides, environmental carcinogens, PAH, and some chemotherapeutic drugs (including alkylating agents, Doxorubicin, and Vincristein). Functional polymorphisms have been reported in at least three of the genes that code for *GSTs* including *GSTM1*, *GSTT1*, and *GSTP*. Both *GSTT1*, and *GSTM1* genes, exhibited a greater degree of polymorphism, one of them being the complete deletion of the gene that causes the loss of enzymatic activity (Alves et al., 2002). 20-50% of individuals do not express the enzyme due to homozygous deletion and are more susceptible to DNA damage caused by PAH and other mutagens (Strange et al., 2001). The GST gene family might modulate leukemia risk via two potential mechanisms either by mediating the metabolism of specific leukemogens or by directly affecting the redox potential within the cell, protecting DNA from free radical-induced damage.

Polymorphisms within the GST genes were found to be associated with susceptibility to non malignant and malignant diseases including AML, (Alves et al., 2002). Patients with a *GSTs* null genotype were believed to exhibit impaired detoxification of environmental genotoxic agents and chemotherapeutic drugs leading to an increased risk of developing primary and secondary cancers and treatment related complications indicating GST polymorphism might contribute to the susceptibility to t-AML/t-MDS. Children carrying the *GSTM1* null genotype were reported to be at increased risk of developing ALL (Krajinovic et al., 1999; Saadat et al., 2000). Crump et al. (2000) reported no association between the *GSTT1*, *GSTM1* gene deletions and AML. Patients with secondary AML had a slightly higher prevalence of the *GSTT1* and *GSTM1* gene deletions compared with denovo AML patients. Over representation of *GSTM1* null homozygous genotype in the

ALL samples (68.1%) was observed when compared to the control population (49%). The *GSTM1* null genotype was found to be correlated with an increased risk of malignancy (Alves et al., 2002). The null *GSTM1* genotype could be associated with increased risk of acute leukemia.

*[1]School of Chemical & Biotechnology, SASTRA University, Thanjavur, [2]Department of Genetics, Osmania University, [3]Department of Medical Oncology, Nizams Institute of Medical Sciences, Hyderabad, India *For correspondence: sattivishnupriya@gmail.com*

Furthermore, *GSTM1* and *GSTT1* null genotypes were apparently related to response, drug side effects and prognosis of patients with AML.

The present study attempts to identify the role of *GSTM1*, *T1* null genotypes in the development of acute leukemia.

Materials and Methods

294 primary acute leukemia cases comprising of 152 acute lymphocytic leukemia (ALL), 142 acute myeloid leukemia (AML) being treated at NIMS (Nizams Institute of Medical Sciences), Hyderabad were selected for the present study. The age and sex matched control samples were randomly selected from different locations in Hyderabad. Patient's clinical data like WBC count, blast%, platelet count, Hb, LDH, complete remission rate (CR) and disease free survival rate (DFS) was noted from the tumor registry file with the help of medical oncologist. Blood samples from both patients and control group were collected into EDTA vacutainers. Genomic DNA was isolated by using salting-out method (Nuremberg and Lahari, 1991).

Genotyping of GSTM1 and GSTT1 polymorphism

PCR was performed using 150-200ng of genomic DNA, 20 pmol/l of each primer (see Table 1), 200μmol/l of dNTPs, 20 mmol/l of Tris HCl, 50 mM of KCl, 2.5 mmol/l of MgCl2, 1U of Taq DNA polymerase. The PCR cycling conditions consisted of initial denaturation at 94^{0}C for 3 minutes followed by 35 cycles of denaturation at 94^{0}C for1minute, annealing at 60^{0}C for 1 minute, extension at 72^{0}C for 2 minutes and final extension at 72^{0}C for 5 minutes. Based on the presence or absence of 219bp and 480bp (see Figure 1), the genotypes were determined as *M1T1*, *M0T0*, *M1T0* and *M0T1*.

Statistical analysis

All the statistical analyses were performed with Statistical Package for the Social Science (SPSS) 15.0. Chi square test was calculated to test the significance of genotype association with the occurrence of acute leukemia and its prognosis. t-test was done to test the significance of association of clinical variables All the p values were two sided and the level of significance was taken as P<0.05.

Results

In the present study, significantly increased frequencies of *GST M* and *T* null genotypes were observed in the both ALL and AML patients as compared to controls (Tables 2 and 3) which indicated that GST null genotypes confer risk to develop acute leukemia This could be due to inefficient detoxification polycyclic aromatic compounds (PAH), environmental pollutants and other mutagens leading to DNA damage (Norappa et al., 2004). *GSTT1* null status was linked to an increased frequency of diepoxy butane induced sister chromatid exchange in culture lymphocytes (Wiencke et al., 1995). The genotype frequencies of GST M0, T0 and *M0T0* did not show association with the sex of the proband in both ALL and AML (Table 4).

With respect to age at onset, increase in the frequency of M0 null genotype was observed in ALL patients with late age at onset of >20 years (67.4%) and in AML patients with early onset <30 years as compared to corresponding age groups. However, double null genotype frequency (*M0T0*) was elevated in ALL females as compared to ALL males.

There was no significant variation in clinical variables of ALL and AML patients with M0 genotype. But patients with T0 genotype had significant leukocytosis, increased

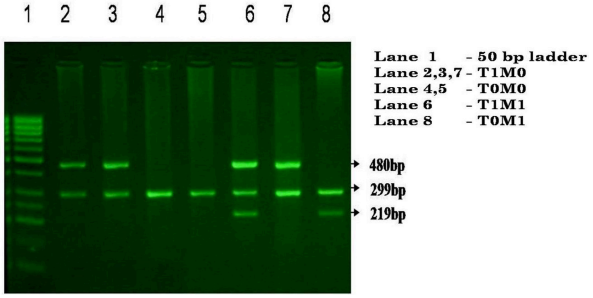

Figure 1. Gel Photograph of GST1M1 Polymorphism

Table 1. GSTM1 and GSTT1 Genotyping was Carried Out by Multiplex PCR using Gene Specific Primers

Gene	Primer Sequences	PCR Product Size
GSTM1	5'-GAA CTC CCT GAA AAG CTA AAG C-3'	219 bp
	5'-GTT GGG CTC AAA TAT ACG GTG G-3'	
GSTT1	5'-TTC CTT ACT GGT CCT CAC ATC TC-3'	480 bp
β- globin	5'-TCA CCG GAT CAT GGC CAG CA-3'	299 bp
(Internal	5'-ACA CAA CTG TGT TCA CTA GC-3',	
Control)	5'-CTC AAA GAA CCT CTG GGT CC-3'	

Table 2. Genotype Distribution of GST M0T0 Polymorphism in Acute Leukemia and Controls

GST M0T0		M0	T0	T0M0
		No %	No %	No %
ALL	(152)	89 (58.6)*	38 (25.0)*	19 (12.6)*
AML	(142)	90 (63.4)*	57 (40.1)*	35 (24.6)*
Controls	(251)	94 (37.5)	39 (15.5)	20 (8.0)

*	M0			T0			M0T0		
	χ^2	df	p	χ^2	df	p	χ^2	df	p
ALL vs Controls	17.006	1	0.000*	5.484	1	0.019*	25.449	3	0.000*
AML vs Controls	24.491	1	0.000*	29.737	1	0.000*	50.165	3	0.000*
Cases vs Controls	29.743	1	0.000*	20.549	1	0.000*	52.707	3	0.000*

Table 3. Genotype Distribution of *GST* M0 T0 Polymorphism and Sex and GST M0T0 polymorphism and Age at onset in Acute Leukemia

Disease		Total	M0	T0	T0M0
			No %	No %	No %
GST M0T0 Polymorphism and Sex in Acute Leukemia					
ALL	Males	105	59 (56.2)	26 (24.8)	10 (9.6)
	Females	47	30 (63.8)	12 (25.5)	9 (19.1)
AML	Males	86	59 (68.6)	37 (43.0)	22 (25.6)
	Females	56	30 (55.6)	20 (37.0)	13 (24.1)
GST M0T0 polymorphism and Age at onset in Acute Leukemia					
ALL	10	43	23 (52.3)	11 (25.0)	5 (11.6)
(years)	10-20	65	37 (56.9)	16 (24.6)	8 (12.3)
	>20	44	29 (67.4)	11 (25.6)	6 (14.0)
AML	<20	25	18 (72.0)*	11 (44.0)	6 (24.0)
(years)	20-30	52	39 (75.0)*	20 (38.5)	17 (32.7)
	>30	63	32 (50.8)	26 (41.3)	12 (19.0)

*p<0.05 is significant

Table 4. Mean Values of Clinical Variables with Respect to GSTM1 Polymorphism in ALL Group and AML Group

Clinical variables	ALL Group					AML Group				
	M1		M0		Total	M1		M0		Total
	Mean±SE	N	Mean±SE	N		Mean±SE	N	Mean±SE	N	
Mean Age	14.27±1.16*	63	16.80±1.09	89	152	36.39±2.22	51	30.04±1.59 *	89	140
Mean WBC(Thousand)	50.23±8.91	63	54.69±7.73	89	152	39.97±9.78	51	58.69±8.40	89	140
Mean blast%	46.78±4.18	63	51.22±3.54	89	152	57.33±3.85	51	61.63±2.82	89	140
Mean platelet count(lakhs)	0.77±0.07	63	0.91±0.08	89	152	1.13±0.20	51	0.93±0.12	89	140
Mean HB	8.93±0.30	63	8.73±0.30	89	152	8.41±0.31	51	8.11±0.26	89	140
Mean LDH	719.79±74.57	63	859.7±79.71	89	152	465.80±45.54	51	494.92±38.65	89	140
Mean DFS	30.60±2.94	58	25.51±1.75	81	139	12.04±2.55	24	10.97±0.93	59	83

*p<0.05 is significant

Table 5. Mean Values of Clinical Variables with Respect to GSTT1 Polymorphism in ALL Group and AML Group

Clinical variables	ALL Group					AML Group				
	T1		T0		Total	T1		T0		Total
	Mean±SE	N	Mean±SE	N		Mean±SE	N	Mean±SE	N	
Mean Age	15.35±0.902	114	16.95±1.754	38	152	32.20±1.71	83	32.58±2.08	57	140
Mean WBC(Thousand)	43.98±5.63	114	79.426±15.4	*38	152	29.59±4.75	83	84.32±13.16 *	57	140
Mean blast%	45.16±3.08	114	62.05±5.124 *	38	152	55.42±3.02	83	66.11±3.32 *	57	140
Mean platelet count(lakhs)	0.88±0.068	114	0.763±0.112	38	152	1.14±0.16	83	0.80±0.11	57	140
Mean HB	8.84±0.23	114	8.75±0.502	38	152	8.11±0.27	83	8.38±0.30	57	140
Mean LDH	748.04±63.87	114	962.76±114.23	38	152	444.66±39.96	83	542.05±42.73	57	140
Mean DFS	29.72±1.97	103	21.67±2.336 *	36	139	12.10±1.41	51	9.97±1.215	32	83

*p<0.05 is significant

Table 6. Mean Values of Clinical Variables in with Respect to GSTM1T1 Polymorphism in ALL Group and AML Group

Clinical variables	*M1T1*		M0T1		M1T0		*M0T0*		Total
	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	
ALL Group									
Mean Age	13.73±1.43	44	16.48±1.16	69	15.53±1.98	19	18.37±2.91	19	151
Mean WBC(Thousand)	39.06±6.59	44	47.50±8.30 *	69	76.10±24.78	19	82.74±19.09 *	19	151
Mean blast%	42.55±4.93	44	47.26±3.99 *	69	56.58±7.62	19	67.53±6.81 *	19	151
Mean platelet count(lakhs)	0.77±0.09	44	0.96±0.09	69	0.78±0.13	19	0.74±0.18	19	151
Mean HB	9.22±0.34	44	8.60±0.32	69	8.27±0.59	19	9.22±0.81	19	151
Mean LDH	611.50±65.37	44	842.46±95.47*	69	970.58±186.73	19	954.95±137.03*	19	151
Mean DFS	34.55±3.98	40	26.63±1.95*	62	21.83±2.42	18	21.50±4.07 *	18	138
AML Group									
Mean Age	36.17±2.72	29	30.07±2.14	54	36.68±3.77	22	30.00±2.37	35	140
Mean WBC(Thousand)	17.29±2.83	29	36.19±7	54	69.86±20.95*	22	93.41±16.97 *	35	140
Mean blast%	48.59±5.25	29	59.85±3.62	54	68.86±4.7 *	22	64.37±4.55 *	35	140
Mean platelet count(lakhs)	1.24±0.32	29	1.09±0.19	54	0.99±0.21	22	0.68±0.12	35	140
Mean HB	8.24±0.43	29	8.04±0.35	54	8.62±0.47	22	8.22±0.39	35	140
Mean LDH	398.79±57.60	29	469.30±53.14	54	554.14±70.45	22	534.46±54.47	35	140
Mean DFS	14.00±3.61	16	11.23±1.24	35	8.13±2.22	8	10.58±1.44	24	83

*p<0.05 is significant

Table 7. GSTM1 and GSTT1 Polymorphism and Complete Remission Rates in ALL and AML Groups

	GSTM1					GSTT1				
	M1		M0		Total	T1		T0		Total
	n	%	n	%		n	%	n	%	
ALL										
CR+VE	58	42.0	80	58.0	138	102	73.9	36	26.1	138
CR-VE	2	50.0	2	50.0	4	3	75	1	25	4
	χ^2-0.101; df-1, (p-0.750)					χ^2-0.002; df-1, (p-0.961)				
AML										
CR+VE	18	28.6	45	71.4	63	41	65.1	22	34.9	63
CR-VE	16	41.0	23	59.0	39	22	56.4	17	43.6	39
	χ^2-1.681; df1, (p-0.195)					χ^2-0.767; df-1, (p-0.381)				

*p<0.05 is significant

Table 8. GSTM1T1 Polymorphism and Complete Remission Rates in ALL and AML Groups

	M1T1		M0T1		M1T0		M0T0		Total
	n	%	n	%	n	%	n	%	
GSTM1T1									
ALL									
CR+VE	40	29.2	61	44.5	18	13.1	18	13.1	137
CR-VE	1	25	2	50	1	25	0	0	4
	χ^2-0.981; df-3, (p-0.806)								
AML									
CR+VE	13	20.6	28	44.4	5	7.9	17	27	63
CR-VE	11	28.2	11	28.2	5	12.8	12	30.8	39
	χ^2-2.956; df-3, (p-0.399)								

*p<0.05 is significant

blast % and reduction in mean DFS. When both patients with deletion of both GST M and T were analyzed (*GSTM0T0*) for various clinical parameters, the results were similar to those observed with respect of T0 genotype indicating that absence GST is associated with poor

prognosis. This might be due to inefficient metabolism of chemotherapeutic agents leading to lack of drug response. Further, it may be observed that patients with both M and T alleles (*M1T1*) exhibited favorable clinical parameters when compared to those with *M0T0* genotype. The deletion of M or T genes is significantly associated with reduction in disease free survival rate indicating the importance of GST enzymes in the metabolism of chemotherapy agents. The data on CR failed to reveal any significant contribution with GST gene deletion which could be due to limited available data on CR.

It was reported that the adult AML patients with GSTM null genotype had a trend towards a poorer survival than those with M1 allele, but no such effects for *GSTT1* and *GSTP* genotypes were reported (Autrup et al., 2002). Barragan et al. (2007) reported the probability of DFS was significantly diminished in patients with GSTM null genotype compared to patients with undeleted *GSTM1*. The absence of GSTM enzyme (*GSTM0*) might predispose to leukemia and also influence the clinical variables specially associated with reduced disease survival. Zhijin et al. (2008) reported that AML Patients with deletions of *GSTM1* or GSTT or both had a lower probability to achieve CR on induction therapy and shorter survival as compared to patients with intact GST genes. In a systemic review and Meta analysis of 30 published case control studies, it was suggested that *GSTM1* and GSTT, polymorphism appeared to be associated with a modest increase in the risk of acute lymphoblastic leukemia (Zhang et al., 2005). Voso et al. (2009) also reported that *GSTT1* null genotype and *GSTM1* null genotype predict or poor response indirection chemotherapy and in consequently to shorter overall survival (OS) in adult AML patients.

Discussion

In conclusion, absence of both *GST M & GST T* might confer risk to develop ALL or AML. The absence of GST enzyme might lead to oxidative stress and subsequently DNA damage resulting in genomic instability, the hall mark of acute leukemia. The GST enzyme deficiencies might also exert impact on clinical prognosis leading to poorer DFS. Hence the GST genotyping can be made mandatory in management of acute leukemia so that more aggressive therapy such as allogenic stem cell transplantation can be planned in the case of patients with null genotype.

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References

Alves S, Amorim FF, Norton L, et al (2002). The *GSTM1* and *GSTT1* genetic polymorphisms and susceptibility to acute lymphoblastic leukemia in children from north Portugal. *Leukemia*, **16**, 1565-7.

Autrup H (2000). Genetic polymorphisms in human xenobiotic metabolizing enzymes as susceptibility factors in toxic response. *Mutant Res*, **464**, 65-76.

Barragan E, Collado M, Cervera J, et al (2007). The GST deletions and NQO1*2 polymorphism confers inter individual variability of response to treatment in patients with acute myeloid leukemia. *Leukemia Res*, **31**, 974-53.

Boyer TD (1989). The glutathione S-transferases: an update. *Hepatology*, **9**, 486-96.

Cludio LS, Cynara GB, Jose Pereirs de MN, et al (2008). Polymorphisms in the glutathione S-transferase theta and mu genes and susceptibility to myeloid leukemia in Brazilian patients. *Genetics and Molecular Biology*, **31**, 39-41.

Crump C, Chen C, Appelbaum FR, et al (2000). Glutathione S-transferase theta 1 gene deletion and risk of acute myeloid leukemia. *Cancer Epidemiol Biom Prev*, **9**, 457-60.

Dirksen U, Moghadam Ka, Mambetova H, et al (2004). Glutathione S- transferase theta 1 gene (*GSTT1*) null genotype is associated with an increased risk for acquired aplastic anemia in children. *Pediatr Res*, **55**, 466-71.

Hoban PR, Robson CN, Davies Sm, et al (1992). Reduced topoisomerase II and elevated alpha class glutathione S-transferase expression in a multidrug resistant CHO cell line highly cross-resistant to mitomycin C. *Biochem Pharmacol*, **23**, 685-93.

Krajinovic M, Labunda D, Richer C, et al (1999). Susceptibility to childhood acute lymphoblastic leukemia: influences of CYP1A1, CYP2D6, and *GSTT1* genetic polymorphisms. *Blood*, **93**, 1496-501.

Norppa H, Barnette P, Scholl R, et al (2004). High-throughput detection of glutathione S-transferase polymorphic alleles in pediatric cancer population. *Cancer Epidemiol Biomark Prev*, **13**, 304-13.

Lahiri DK, Nurnberger JI Jr (1991). A rapid non-enzymatic method for the preparation of HMW from blood RFLP studies. *Nucleic acid Res*, **19**, 5444.

Sadat I, Saadt M (2000). The glutathione S-transferase polymorphisms and susceptibility to acute lymphocytic leukemia. *Cancer Letter*, **158**, 43-5.

Seedhouse AC, Faulkner R, Ashraf N, et al (2004). Polymorphism in genes involved in homologous recombination repair interacts to increase the risk of developing acute myeloid leukemia. *Clinical Cancer Res*, **10**, 2675-80.

Smith MT, Wang Y, Skibola cf, et al (2002). Low NAD (P) H: quinine oxidoreductase activity is associated with increased risk of leukemia with MLL translocation in infants and children. *Blood*, **100**, 4590-3.

Strange RC, Spiteri MA. Ramachandran S, Fryer AA (2001). Glutathione-S-transferase family of enzymes. *Mutat Res*, **482**, 21-6.

Voso MT, D'Alo F, Putzulu R, et al (2002). Negative prognostic value of glutathione S- transferase (*GSTM1* and *GSTT1*) deletions in adult acute myeloid leukemia. *Blood*, **100**, 2703-7.

Wiencke JK, Pemble S, Ketterer B, Kelsey KT (1995). Gene deletion of glutathione transferase:correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol Biomarkers Prev*, 4, 253-9.

Zheng Y, Honglin S (2005). Glutathione S- transferase polymorphisms (*GSTM1*, *GSTP*1 and *GSTT1*) and the risk of acute leukemia: a systematic review and meta-analysis. *Eur J Cancer*, **41**, 980-9.

Zhijian X, Lin Y, Zefeng XU, Yue Z, Liang L, (2008). Glutathione S-transferase (*GSTT1* and *GSTM1*) gene polymorphisms and the treatment response and prognosis in Chinese patients with denovo acute myeloid leukemia. *Leukemia Res*, **32**, 12.