Original Research Article

Genetic polymorphisms of GSTM1 and GSTT1 genes and susceptibility to Acute Lymphoblastic Leukemia in the Yemeni population

Abstract

Background: GSTs (glutathione S-transferases) are enzymes that are well known for their capacity to detoxify hazardous substances. Previous research has found a link between GST polymorphisms and acute lymphoblastic leukaemia (ALL). The outcomes differed depending on the study and population.

Objectives: to analyze the relation between polymorphisms of glutathione s-transferase Mu (GSM1) and glutathione s-transferase theta (GSTT1) genes and susceptibility to acute lymphoblastic leukemia (ALL).

Methods: a total of 115 patients with ALL who attended oncology centers in Yemen and 140 unrelated apparently healthy individuals as the control group were recruited in this case-control study. DNA was extracted from EDTA venous blood samples and analyzed by Multiplex PCR for detection of the polymorphic deletion of the GSTT1 and GSTM1 genes.

Results: The GSTT1 null genotype were found to increase the risk of acute lymphoblastic leukemia, (OR=2.649, 95%CI=1.589-4.416, p=0.000). The GSTM1 null genotype was not significant (p=0.076), however is risk for ALL (OR=1.481, 0.902-2.431). The combination effects of GSTT1null and GSTM1null were associated with the susceptibility to acute lymphoblastic leukemia (OR 3.396, 95% CI 1.832-6.297) (p =0.000).

Conclusion: Susceptibility to ALL appears to be significantly related to GSTT1 null polymorphism but not to GSTM1 polymorphism in Yemeni population.

Keywords; GSTM1, GSTT1, Yemen, Acute lymphoblastic leukemia, Glutathione *S*-transferees, Genetic polymorphism

Introduction

Leukemia is a type of blood cancer that develops when hematopoietic cells in the bone marrow (BM) undergo neoplastic transformation and clonal growth, resulting in a significant number of neoplastic cells in the peripheral blood.

The reactive species generated by carcinogens mediate this damage and this can be the result of oxidative metabolism or environmental mutagens[4]. S-transferase GSTs are second stage enzymes that stimulate the coupling of mutagens to glutathione, facilitating their solubility in water and excretion in urine[5]. Among these, are glutathione-S-transferase (GST) M1 and T1. These are involved in the detoxification and metabolism of reactive oxygen species, carcinogens and xenobiotics. Genetic variations in this enzyme family are found to be associated with high risk for development of some primary cancers and cancers secondary to chemotherapy[6,7].

In GSTM1 and GSTT1 , the genes encoding the enzymes are polymorphic. Polymorphisms in GSTM1 and GSTT1 genes decrease the activity of the enzymes leading to elevated susceptibility to environmental toxins[7,8].

Methionine and Folate metabolism have important role in the synthesis of DNA and process of methylation. In the folate-dependent enzymes , the polymorphisms in the genes may influence cancer susceptibility[9,10].

Glutathione S-transferases (GSTs), consists of three super families': the cytosolic, mitochondrial, and microsomal also known as MAPEG proteins[11,12]. GSTM1 and GSTT1 genotype status with various malignant tumors such as smoke-induced lung cancer, breast , digestive or bladder cancer[13,14,15]. An increased risk for individuals with GST genotypes with decreased level of enzyme activity was observed in some studies[13-16]. GSTs can also confer resistance to cytotoxic agents used to treat cancer [17,18]. Unlike the role of GSTs in carcinogenesis in the environment, GST genotypes that result in low enzyme activity can be beneficial for people undergoing chemotherapy for oncology because poor detoxification improves the effectiveness of chemotherapy.

The common drugs for anticancer like chlorambucil, cyclophosphamide, melphalan, and steroids are substrates for GSTs [18-19]. Indirect evidence has been found of the role of GSTs in modifying the effect of the drug by deactivating drug hydroperoxides or other reactive oxygen-containing types of doxorubicin, mitomycin C and cisplatin [20-21].

Exposure to exogenous and endogenous toxic substances can cause genetic changes and, therefore, increase cancer susceptibility[22]. Environmental toxic factors for cells and genetic toxicity (like ionising radiation) are claimed to increase the risk of causing cancers[23]. Xenobiotic metabolising (XME) enzymes are the first lines of defense against environmental carcinogens.

Several studies recently examine different genes polymorphism inside GSTs family and they found significance association between the polymorphism and cancer risk and prognosis of the disease [24-27]. Some authors recommend assays for such polymorphisms as investigative protocol in cancer patients [28].

The researchers tested the connection of GSTT1 and GSTM1 gene polymorphisms with susceptibility to acute lymphoblastic leukaemia in a sample of Yemeni people at cancer centres in Yemen (Taiz, Aden, and Hadramout). Specimens were examined at Aden's Alsadaqa teaching hospital. Sudan's University of Khartoum conducted a molecular experiment.

Materials and methods

Study population

Patients diagnosed with ALL between 2015 and 2018 who visited oncology centres were recruited to participate in the trial after giving their informed consent. As controls, 140 seemingly healthy persons of similar age and gender were used.

DNA extraction

From EDTA blood sampling , the DNA was extracted by using DNA purification kit (G-spin™ Total DNA extraction kit protocol Intron Biotechnology). DNA was quantified by nanodrop and stored at -20° C.

Genotyping of GSTT1 and GSTM1 polymorphism

Multiplex polymerase chain reaction (conventional) was used for detection of the polymorphic deletion of the GSTT1and GSTM1. Briefly, this consisted of applying the PCR in a volume of up to 20 µl, this includes 1 µl genomic DNA, 1µl each primer and 15µl distilled water, ready to load master mix (Maxime TM premix kit (i-Taq). PCR reaction conditions included initial denature at

94°C for 3 minutes, taken after 30 cycles at 94 ° C for 30 seconds, 60.5 ° C for 30 seconds, 72 ° C for 50 minutes and final expansion at 72 ° C for 10 minutes

The products of PCR were analyzed on a 2% Agarose gel. After buffering and staining with 3 µl ethidium bromide the visualization was completed by gel documentation system. A 100 bp DNA ladder *(Vivantis 100 pb plus, 0.1 µg /µl)* was run with each batch of patients' and control samples. Genetic profiling (null genotypes) was demonstrated by not? using 480 bp separately for GSTT1 and 219 bp for GSTM1 PCR elements, using GSTP1 (436 bp) as a positive control.

This result of genotyping obtained does not distinguish between GST homozygote (+/+) and heterozygotes (+/ –) (Figure 1).

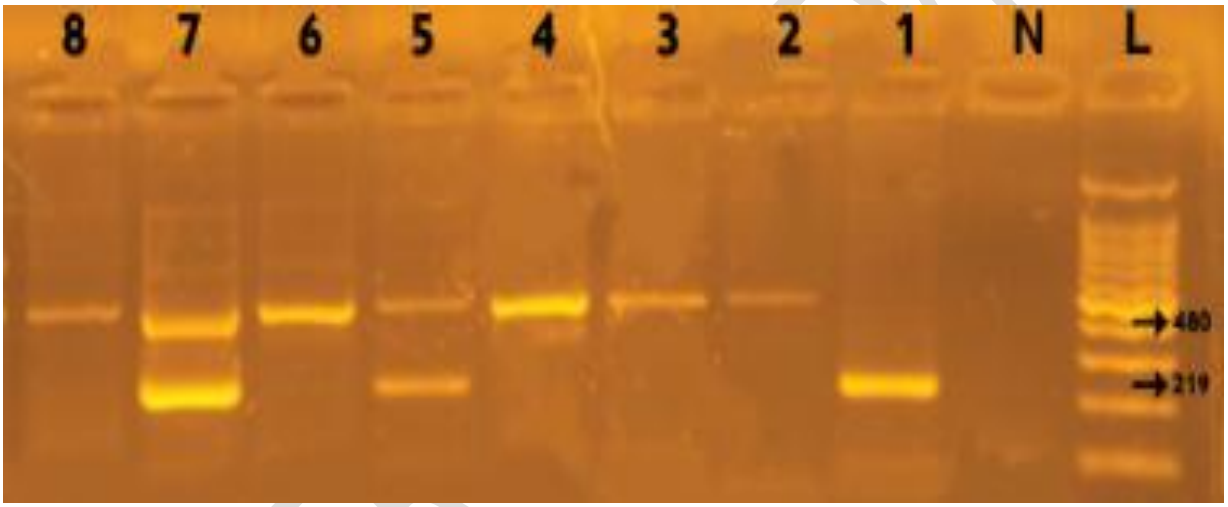

Figure 1. Amplified DNA run in 2% agarose gel electrophoresis

Lane L DNA ladder: MW 100-1500 bp fragments, lane1: 1 fragment at 219bp indicates the presence of *GSTM1* only, lane5 and 7: 2 fragments at 219 bp and 480 bp indicates the presence of both *GSTM1* and *GSTT1*, respectively. Lane 2,3,4,6 and 8: show 1 fragment at 480 bp indicates the presence of *GSTT1* only and lane N: show the absence of both 219 bp and 480 bp indicate homozygous deletion of *GSTT1* and *GSTM1*.

Statistical Analysis

Demographic data were analyzed to obtain the mean, the standard deviation and the probability (P value) between patients and control was introduced in to SPSS program (version 20), the

analysis data tabulated in statistical tables and graphs. A P-value of ≤0.05 was considered statistically significant. Odd ratios were estimated for each variable. Logistic regression analysis to estimate the risk of developing ALL according to demographic data, with the 95% confidence limits.

Results

Table 1 summarises the demographic distribution of the study groups. Patients were divided into four groups, ranging in age from one to sixty years. The majority of the patients were under the age of ten years, and the differences were statistically significant (P value =0.000). Whereas, gender, occupation and education were non significantly difference with P value 0.574, 0.844, 0.852 respectively. see (Table 1)

Table 1. Demographic variables of all patients and controls

		Patients (no =115)	Controls (no=140)	P value
Age group	<10year	70(60.9%)	30(21.4%)	0.000
	11-20 year	29(25.2%)	98(70%)	
	21-30 year	13(11.3%)	11(7.9%)	
	>30 year	3(2.6%)	1(.7%)	
Gender	Female	49(42.6%)	61(43.6%)	0.574
	Male	66(57.4%)	79(56.4%)	
Occupation	Without	110(95.7%)	128(91.4%)	0.844
	Farmer	2(1.7%)	0(0%)	
	Military	2(1.7%)	11(7.9%)	
	House ladies	1(.9%)	1(0.7%)	

Education	Illiterate	52(45.2%)	23(16.4%)	0.852
	Primary school	43(37.4%)	102(72.9%)	
	Secondary school	20(17.4%)	15(10.7%)	

The GSTT1 null genotype in patient group was higher (55.7%) while in controls group (32.1%). Significant difference was found between the distribution of genotype frequency among cases and controls (P=0.00). Risk of ALL by GSTT1 null genotype was statistically significant (OR=2.649, 95%CI=1.589-4.416, P=0.00). The difference between ALL patients and controls regarding GSTM1 null was not significant (p=0.076). However, the GSTM1 null genotype was risk for ALL (OR=1.481,0.902-2.431) (Table 2).

Table 2.GSTT1, GSTM1 genotypes in Yemeni acute lymphoblastic leukemic patients

Gene	Genotype	Patients 115	Control group 140	P value	OR	95%CI	
						Lower	Upper
GSTT1	Null	64(55.7%)	45(32.1%)	0.00	2.649	1.589	4.416
	Present	51(44.3%)	95(67.9%)				
GSTM1	Null	63(54.8%)	63(45%)	0.076	1.481	0.902	2.431
	Present	52(45.2%)	77(55%)				

The GSTT1null /GSTM1 null genotype was significantly difference between ALL patients and control, it was 40(34.8%) in ALL patients and 19(13.6%) in controls {P value =0.000, odds ratio (OR) 3.396, 95% confidence interval (CI) 1.832-6.297 (Table 3).

Table 3.The combined effects of GSTT1 and GSTM1 null genotypes

Gene	Patients 115	Control group 140	P value	OR	95% CI	
					Lower	Upper
GSTT1null /GSTM1 null	40(34.8%)	19(13.6%)	0.000	3.396	1.832	6.297

Discussion

Toxins, whether exogenous or endogenous, can cause gene changes that enhance vulnerability to cancer formation, such as ALL [29]. Important metabolising enzymes like GSTs are involved in the detoxification of xenobiotics or endogenous chemicals brought into the body [30,31]. Hence alterations in those enzyme may lead to absence of the enzymes like in GSTT1 and GSTM1 *null* Polymorphism[32,33]. Several studies examined different genes polymorphism inside GSTs family have been done on different types of cancer like ALL, however, the results are variable. For the GSTT1gene, the zero ? genotype (14.4% ALL and 8.5% controls) was not statistically significant for GSTT1gen[34]. Likewise, found no association between nullGSTM1, nullGSTT1 pattern frequencies and ALL in childhood[35]. Other study report there were no association between the risk of childhood ALL and the value of null GSTM1 and null GSTT1 genetics[36]. A study by Aydin - Sayitoglu et al. (2006) on the Turkish population showed there was no association between the recurrence of nullGSTM1 and the GSTT1 genotypes and the possibility of the development of ALL in childhood[37].

In contrast, Dunna et al. (2013) found the frequency increase significantly in the GSTM1-free genotype, the GSTT1-free genotype and the GST-free genotype (T null M null) in all cases compared to the controls[38]. In Egypt, null GSTT1 and GSTM1 genotypes was connected with the risk of developing ALL[39]. A stratification analysis showed there is association between acute

childhood leukemia in the Asian group and the risk of a GSTM1 polymorphism (OR = 1.94; 95% CI, 1.53-2.46). Regarding the significant association between GSTT1 polymorphism and the risk of acute childhood leukemia was found in the Asian subgroup[40].

Several studies have looked at the effects of GSTs on the risk of developing some hematological malignancies . A few studies have linked the genetic differences in GST and risk of AML development. The relationship between risk of AML development and the GSTT1 and the null GSTM1 genotypes was reported by some studies in Caucasians and East Asia[41-43] . The relationship between the GSTM1null genotype and CLL risks was reported by Yuille et al. (2002)[44] . Several studies have reported the associated risk between null GSTM1and NHL[45,46]. In another study by Kerridge et al., 2002 showed that the nullGSTT1 genotype association with high risk of Non-Hodgkin's lymphoma[47]. Differences between these results can affect the relation between genetic polymorphism and risk of leukemia of these two conditions. The first is that the impacts of the GSTM1 and GSTT1 genotypes on genotypes vulnerability to leukemia development vary among the population. The second is that due to certain interactions between genes-genes and environment and genes. The null genotype free of enzymatic activity was reported in GSTM1gen in 38-62% of the Caucasians and the nullGSTT1 genotype was reported in the Caucasian population with a frequency of 27-73% [48].

Our study is the first to evaluate the association of these genes in Yemeni individuals. In our study we observed that the frequency of people with a GSTT1-null genotype was higher in ALL patients (55.7%) in comparison to to control groups (32.1%). Significant difference was found between the genotype frequency distribution among cases and controls (P value =0.000). The risk of ALL by GSTT1 null genotype was statistically significant (OR=2.649, 95%CI=1.589-4.416). Similar results were indicated in north Indian studies done by (Moulik *et al*., 2014)[49] (P value =0.0007) (OR=2.54, 95%CI=1.50-4.32), meta-analysis on 7 Asian studies (OR=1.63, 95%CI=1.32-1.99 and in Egypt by (Swellam et al., 2016)[39] (P value =0.0001) (OR=0.523, 95%CI=0.9-1.07). In contrast the results were disagreed with many previous studies. In Turkish case -control study (P value =0.71) (OR=0.90, 95%CI=0.51-1.57)[27]. In Brazil, investigators found that the frequencies of null GSTT1 genotype were not related with the hazard of creating childhood ALL[36]. In expansion. Another study found an association of GSTM1 with childhood ALL, though there was no association with GSTT1 genotype[49]. Moreover no critical distinction

was found between the ALL patients and the control group due to frequency of the GSTT1genotype (for the null genotype, in patients with ALL 19.2% and 25.5% for control group)[50].

These discrepancies can be attributed to the genetic susceptibility of developing ALL among the different populations and or due to the genotypic polymorphism with the environmental exposure to carcinogenic hazardous[27].

In this study, the GSTM1 null in patients with ALL and control group was not significant (p=0.076), although most of them found the null genotype was more frequent in patients than in control 54.8% and 45%, respectively. However, the GSTM1 null genotype was risk for ALL in Yemeni individuals (OR=1.481, 0.902-2.431). But this result remains doubtful regarding most of studies, because most of them found that association were observed. Our results were agreed with (Al-Eitan et al., 2016)[51] from Jordan (P value=0.57), (Moulik et al., 2014)[49] in north India (P value=0.206) (OR=1.38, 95%CI=0.85-2.24), and (Canalle et al., 2004)from Brazil[36].

The combined effect of the GSTT1null /GSTM1 null was highly significant between patients with ALL and control 40(34.8%) in ALL patients and 19(13.6%) in controls {P value =0.000, odds ratio (OR) 3.396, 95% confidence interval (CI) 1.832-6.297} These results agreed with combined analysis of GSTP1 and GSTM1 (IIe/Val)/(Val/Val) genotype done by (Suneetha et al., 2008)[52] showed increase risk for ALL patients significantly (OR=2.78: 95 CI=1.16-6.69) and there were direct association and proportion between GSTT1and GSTM1 null genotype and ALL.[49]

Conclusions: This study analyzed the relation between polymorphisms of glutathione s-transferase Mu (GSM1) and glutathione s-transferase theta (GSTT1) genes and susceptibility to acute lymphoblastic leukemia (ALL) and concluded there is significant association between the GSTT1 null polymorphisms and ALL development in Yemen but the association with GSTM1 null genotype was not significant.

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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