Original Research Article

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

Abstract

Background: Glutathione *S*-~~transferees~~ transferases (GSTs) are enzymes best known for their ability in detoxification of toxic substance. Previous studies reported the association in the polymorphisms of GSTs with the acute lymphoblastic leukemia (ALL). The results varied between studies and population.

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

Methods: a total of 115 patients with ALL ~~attended~~ who attended oncology centers in Yemen and 140 unrelated apparently healthy individuals as the control group were ~~involved~~ recruited in ~~a~~ this case-control study. DNA was extracted from ~~collected~~ 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.Value*~~*p*=0.000). ~~but t~~The GSTM1 null genotype was not significant (*p*=0.076), however is risk for ALL (OR=1.481, 0.902-2.431) it is unclear what you actually mean and how you concluded. 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) (~~*Value*~~ *p* =0.000).

Conclusion: ~~There is significant association between the GSTT1 null polymorphisms and ALL development in Yemen but the GSTM1 null genotype was not significant.~~Susceptibility to ALL

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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~~ Leukemia refers to ~~Blood~~ blood disorders caused by neoplastic transformation and clonal expansion of hematopoietic cells in the bone marrow (BM) causing large number of neoplastic cells in the peripheral blood[1]. ~~Cancer is a disease characterized by the development of abnormal cell growth that divide in an uncontrolled way and has the ability to infiltrate and spread and destroy and damage normal body tissues, often develop mutations in their DNA, but they have the ability to repair most of these mutations, some times are not able to repairs and the cells often die. However, certain mutation are not repaired, causing the cells to grow and become cancerous. Mutation may cause cancer cells to live beyond a normal cell lifespan this lead to the cancerous cells to accumulate[2,3].~~

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

In GSTM1 and GSTT1 , the genes encoding the enzymes are polymorphic~~,~~. ~~and polymorphisms~~ Polymorphisms in GSTM1 and GSTT1 genes ~~will decreased~~decrease the activity of the enzymes ~~which~~ leading to elevated susceptibility to environmental ~~as well as other~~ toxins[7,8].

Methionine ~~AND~~ 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].

~~Ligandins is named Glutathione S-transferases (GSTs), which comprise a family of prokaryotic and eukaryotic phase II metabolic isozymes. For the purpose of detoxification it is characterized by its capacity to catalyze low frame glutathione (GSH) conjugation to xenobiotic substrates.~~ 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 ~~adriamycin~~doxorubicin, mitomycin C~~,~~ and cisplatin ~~and~~ [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 (~~especially~~ like ~~ionization, radiation, and the like substances~~ionising radiation) are claimed to increase the risk of ~~developing~~ causing ~~all conditions~~cancers[23]. Xenobiotic ~~metabolism~~ metabolising (XME) enzymes are ~~one of~~ the first lines of defense against environmental ~~chemicals~~carcinogens.

Several studies recently examine different genes polymorphism inside GSTs family and they found significance association between the polymorphism and cancer risk ~~factors~~ and prognosis

of the disease [24-27]~~,~~. ~~they~~ Some authors recommend~~ed being~~ assays for such polymorphisms as investigative protocol ~~for~~ in cancer patients [28].

This study was carried out at oncology centers in Yemen (Taiz, Aden and Hadramout) to assess the association of GSTT1 and GSTM1 gene polymorphisims with susceptibility to acute lymphoblastic leukemia in a sample of Yemeni population. Specimens were analyzed in Alsadaqa teaching hospital in Aden. Molecular experiment was carried out at the University of Khartoum, Sudan.

Materials and methods

Study population

This study was conducted on 115 Patients attending the oncology centers who were diagnosed with ALL in the period from 2015- 2018 were invited to take part in the study ~~and~~ after ~~sign~~ an informed consent. ~~and~~ 140 apparently healthy ~~controls~~ age and gender matched individuals ~~to the cases in gender and age~~served as controls.

~~The study includes all Yemeni patients who have confirmed diagnosis of ALL, at any age, both sexes and from different areas, who were attended oncology centers in the study period. The control group were healthy individual who matched to patients in gender and age.~~

DNA extraction

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

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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 ~~Apply~~ 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) ~~lot number 32310250~~. PCR reaction condition~~s includes~~ 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~~. T~~ the visualization ~~will be~~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. ~~The failure to participate in the GSTP1 amplification PCR response was disappointed~~

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

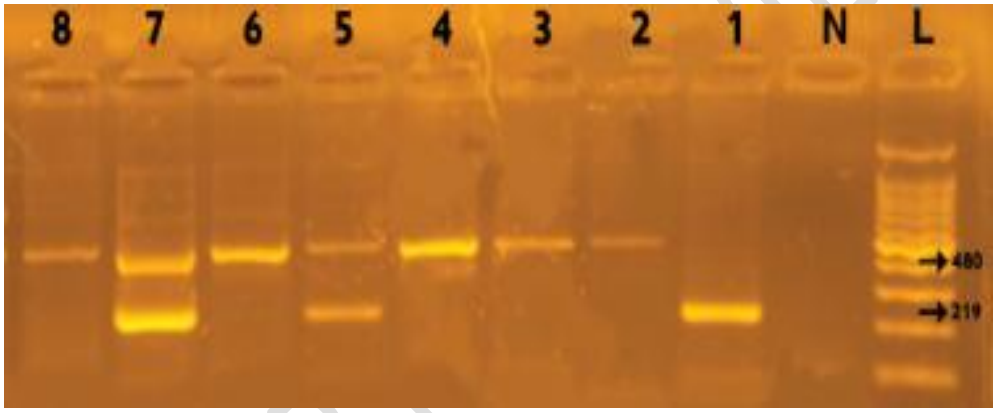

Figure 1. Amplified DNA run in 2% agarose gel electrophoresis

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

~~significant~~ statistically significant ~~when decreased less than 0.05 were~~. 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. ~~P Value of ≤0.05 was considered as statistically significant.~~

Results

The demographic distribution among study groups are summarized in Table 1.Patients were grouped from one year to sixty years in four groups most of them were less than 10 years, and are statistically difference (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)

Of what relevance is gender, age and occupation to genomic data?

Table 1. Distribution of demographic variables of the 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

Exogenous ~~and~~ or ~~Endogenous~~ endogenous toxins may lead to alterations ~~inside~~ of different genes, which may increase susceptibility to cancer development, like ALL[29]. Detoxification of xenobiotics or endogenous compounds introduced into the body, ~~will be done~~is carried out by important metabolizing enzymes like GSTs [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~~ 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 ~~seriousness~~ 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 ~~Yemen and the first of its kind to be conducted among~~ Yemeni individuals. In our ~~recent~~ 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

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expansion~~,~~. Another study found an ~~affiliation of as it were~~association of GSTM1 with childhood ALL, though there was no ~~affiliation watched~~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 ~~the present~~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-~~transferees~~ transferase Mu (GSM1) and glutathione s-~~transferees~~ 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.

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