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Orginal Research Article

Frequencies of GSTP1 (Ile105val) Polymorphism and its Association with Acute Lymphoblastic Leukemia in Yemeni Patients

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Abstract: Background: Glutathione *S*-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-transferase (GSTP1)* Ile105Val *genes and susceptibility to acute lymphoblastic leukemia (ALL).* **Methods:** A total of 115 patients with ALL attended oncology centers in Yemen and 140 unrelated apparently healthy individual as control group were involved in a case-control study. DNA was extracted from collected EDTA venous blood samples and analyzed by PCR-restriction fragment length polymorphism for detection the mutation of *GSTP1* gene. **Results:** The GSTP1 Ile105Val polymorphism were increase the risk of acute lymphoblastic leukemia (p value = 0.005, OR = 1.972, 95%CI=1.194–3.259). The combined effects of GSTT1null, GSTM1null and GSTP1IIe105Val polymorphism were associated with the susceptibility to acute lymphoblastic leukemia (OR 4.125, 95% CI 1.768-9.62) (P.value=0.000). **Conclusion:** The GSTP1 Ile105Val polymorphisms were represent significant associated with ALL development in Yemen (alone or combined with other GSTs).

Keywords: Yemen, Acute lymphoblastic leukemia, Glutathione *S*-transferases (GSTP1 Ile105Val), Genetic polymorphism.

INTRODUCTION

The cause of acute lymphoblastic leukaemia is not known. There are, be that as it may, a couple inclining acquired conditions, and procured chance variables related with a higher occurrence of ALL. Scatters related with chromosomal aneuploidy or instability, for example Bloom disorder, ataxia telengectasia and Down syndrome are all the more ordinarily connected with ALL. So also, introduction to mutagens, for example, ionizing radiation, Benzene or chemotherapy has been implicated.. In any case, most of cases happen sporadically (Pui *et al.,* 2008). Epidemiologic information point to diseases as causal presentation for the improvement of ALL (Greaves *et al.,* 1993).

Glutathione-s-transferases GSTs are a family enzymes of phase II catalyzes the conjugation of mutagenic substances to glutathione which is water soluble and can easily be excreted from the body (Haranatha and Jamil, 2006). Among this Glutathione S-transferase P1 (GSTP1) enzyme are involved inbiotransformation and bioactivation of certain environmental pollutants such as benzo [a] pyrene and other aromatic hydrocarbons (Hengstler *et al.,* 1998).

GSTP1gene has a place with the pi class quality family, situated on chromosome 11q13 (Autrup2000). The primary polymorphism distinguished is an A– G polymorphism at nucleotide 313 in exon 5 of GSTP1gene which prompts an amino acid substitution of isoleucine by valine (val) at 105 amino corrosive position (Ile105Val). This substitution results in three GSTP1genotypes: they are wildtype, isoleucine/isoleucine (Ile/Ile), isoleucine/valine (Ile/Val) heterozygous and valine/valine (Val/Val) homozygous. (Zimniak *et al.,*1994; Johanson *et al.,*1998),

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This study was carried out at oncology centers in Yemen (Taiz, Aden and Hadramout) to assess the association of GSTP1 (Ile105Val) 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 sign an informed consent and 140 apparently healthy controls matched to the cases in gender and age.

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

DNA was extracted from EDTA blood samples using DNA purification kit (G-spin TM Total DNA extraction kit protocol intron biotechnology).DNA was quantified by nanodrop and stored at -20 c.

Genotyping of GSTP1 (Ile105Val) polymorphism

GSTP1 (Ile105Val) polymorphism was determined with a polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP].

The PCR primers were: 5'-GTA GTT TGC CCA AGG TCA AG-3' (F) and 5'-AGC CAC CTG AGG GGT AAG-3' (R). PCR was carried out in a total volume of 20µl. It consists 1µl genomic DNA, 1µl each primer, ready to load master mix (Maxime TM premix kit (i-Taq) and 17µl distilled water. PCR condition includes initial denaturation at 94°C for 3 minutes, followed by 30 cycles at 94°C for 30 second, 60.5°C for 30 second, 72°C for 50 second and a last extension at 72°C for 10 minutes (the same program for GSTT1 and GSTM1). PCR products were analyzed on a 2% Agarose gel stained with 0.3 µg/mL ethidium bromide, and visualized by gel documentation system (to check the presence of 436 pb of GSTP1).Then the PCR product was digested with the restriction endonuclease BsmA1 restriction enzyme {New England Biolabs BsmA1# R0529S}as follow: For each 7 µl of PCR product, 1 µl from 10X NEB buffer and 0.5 µl from BsmA1restriction enzyme were added, then incubated at 37˚C for 20 hrs, followed by incubation at 65˚C for 20 minute to inhibit the enzyme activity. The products are then resolved on 2% agarose gel electrophoresis containing ethidium bromide, then visualized using UV trans illuminator. The amplified part after assimilation with BsmA1 restriction enzyme, will offer ascent to: 2 sections at 329 bp and 107 bp showing the nearness of wild kind (IIe/IIe), appearance of 2 pieces at 222 bp and 107 bp demonstrates the nearness of homozygous freak type (Val/Val), while presence of 3 parts at329 bp, 222 bp and 107 bp shows the presence of heterozygous mutant type (Ile/Val). For quality control, genotyping of the samples were repeated blindly and were indistinguishable to the underlying outcomes.

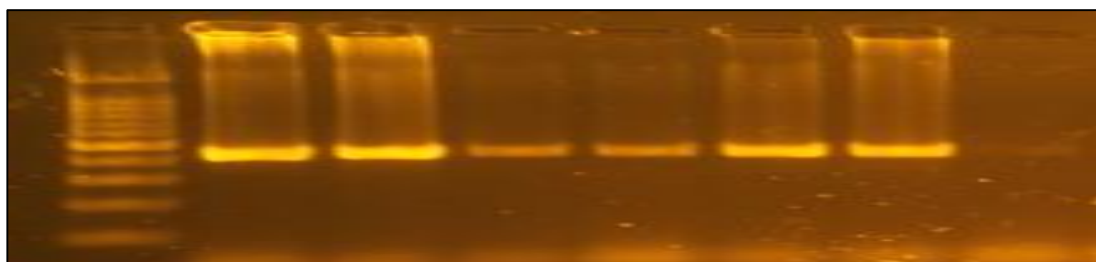

Figure 1. PCR product of GSTP1 gene M, 100 bp ladder molecular weight marker.1ane 1-6 ,436 bp PCR product and lane 7 negative control.

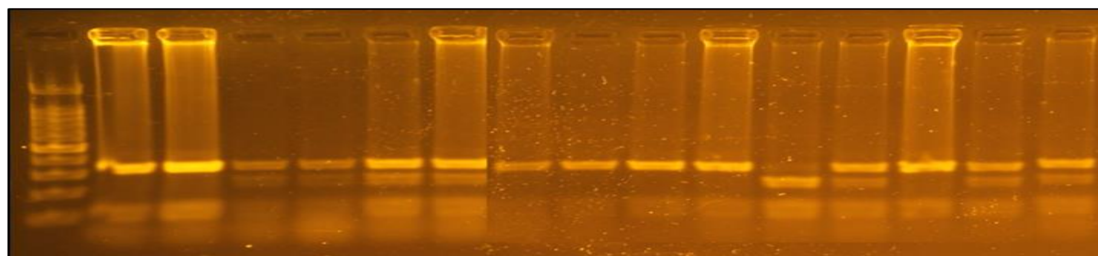

Figure 2. DNA fragment digestion with BsmA1 restriction enzyme

Lane DNA ladder: MW 100-1500 bp fragments, lanes with 2 fragments at 329 bp and 107 bp indicates the presence of wild type (*IIe/IIe*), lanes with 3 fragments at at329 bp, 222 bp and 107 bp indicates the presence of heterozygous mutant type (*Ile/Val*).Laneswith 2 fragments at 222 bp and 107 bp

indicates the presence of homozygous mutant type (*Val/Val*).

Statistical Analysis

Demographic data were analyzed to obtain the mean, the standard deviation and the probability (P value) between patients and control group using Statistical Packages of Social Science (SPSS) software program version 16. The parsons chi-square test was used to compare the genotype distribution between patients and control. P-value less than 0.05 were considered as statistically significant. Odd ratios were estimated for each variable. Logistic regression analysis was used to estimate the risk of developing ALL according to demographic data. P value of <0.05 was considered significant.

RESULTS

The distribution of demographic characterisitcs 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)

Table 1. Distribution of demographic variables of the ALL patients and controls

		Cases(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	Male	66(57.4%)	79(56.4%)	0.574
	Female	49(42.6%)	61(43.6%)	
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%)	

Clinical characteristics of acute lymphoblastic leukemic patients

The most common clinical presentation on ALL patients was fever100%, followed by lymphadenopathy, pallor, splenomegaly and hepatomegaly at 90.40%, 76.50%, 70.40%, and 69.60%, respectively (Figure 3)

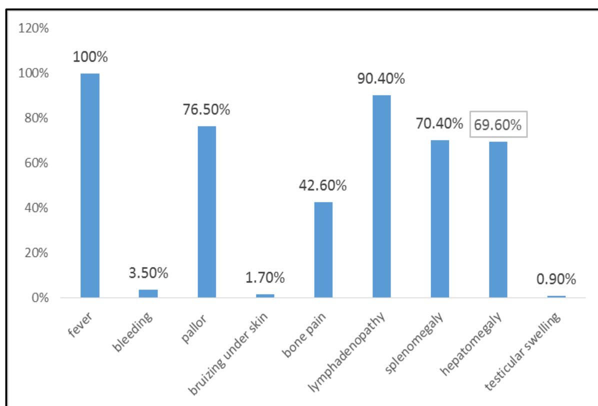

Figure 3. Clinical characteristics of acute lymphoblastic leukemic patients

Hematological parameters in ALL patients and controls

There was significance difference on the mean of WBCs, Hb, MCV, MCH and Platelet's count between patients and controls (P=0.000). While the mean of RBCs and MCHC were none significantly difference between patients and controls (Table2).

Table2. Hematological parameters in ALL patients and controls

Traits	Cases		Controls		P-value
	mean	±SD	mean	±SD	
WBCs	15.3	16.6	7.34	2.86	0.000
RBCs	4.5	0.67	4.67	0.61	0.08
Hb	9.9	2.3	12.5	1.61	0.000
MCV	81.3	6.4	78.6	5.49	0.000
MCH	29.3	2.8	25.9	2.32	0.000
MCHC	31.4	2.9	31.9	1.11	0.089
PLT	189.9	1.1	391.96	96.0	0.000

GSTP1 genotypes in Yemeni acute lymphoblastic leukemic patients and controls

A total of 60% (69) ALL patients have heterozygous (*Ile/Val*) type of GSTP1 Ile105Val polymorphism, 1.7% (2) patients have the homozygous type (*Val/ Val*) and 38.3% (44) have the wild genotype of GSTP1 (*Ile / Ile*). In contrast there were 42.1% (59) control people with heterozygous (*Ile/Val*) type, 2.9%(4)with homozygous type (*Val/ Val*) and 55%(77) with wild genotype of GSTP1 (*Ile / Ile*). GSTP1 Ile105Val polymorphism (*Ile/Val*) and (*Val/ Val*) genotypes frequency was found to be significantly elevated in patient with ALL 61.7%(71) compared to Controls 45%(63) (p value = 0.005, OR = 1.972, 95% confidence interval (CI) 1.194–3.259) (Table3).

Table 3. GSTP1 genotypes in Yemeni acute lymphoblastic leukemic patients and controls

Gene	Genotype	Cases N= 115	Control N=140	P.V	OR	95%CI	
						lower	upper
GSTP1	Heterozygous(IIe/Val)	69(60.0%)	59(42.1%)	0.005	1.972	1.194	3.259
	Homozygous(Val/Val)	2(1.7%)	4(2.9%)				
	Normal(IIe/IIe)	44(38.3%)	77(55%)				

The combined effects of GSTT1, GSTM1 and GSTP1 null genotypes in cases and controls

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}. Among the three genes [(GSTT1 *null*, GSTM1 *null* and GSTP1 (Il105Val)] there was highly significance differences between ALL patients and control 23(20%) in ALL and 8(5.7%) in controls, {odds ratio (OR) 4.125, 95% confidence interval (CI) 1.768-9.626, P value =0.000} (Table 4).

Table4. The combined effects of GSTT1, GSTM1 and GSTP1 null genotypes among cases and controls

Gene	Cases N=115	Control N=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
GSTT1null /GSTM1 null with GSTP1(II105Val polymorphism	23(20%)	8(5.7%)	0.000	4.125	1.768	9.626

Table 5.Relationship between GSTP1 genotypes and risk factors in acute lymphoblastic leukemia patients

Risk factors		GSTP1 in ALL(n=115)			P value
		Wild(II/II)	Heterozygous(II/Val)	Homozygous(Val/Val)	
Family history	Yes	7	11	1	0.649
	No	37	58	1	
Radiation	Yes	4	8	0	0.822
	No	40	61	2	
Smoking	Yes	2	9	0	0.217
	No	42	60	2	
Negative smoking	Yes	1	19	0	0.986
	No	33	50	2	
Infection	Yes	25	37	1	0.720
	No	19	32	1	

Table 6. Relationship between GSTP1 genotypes and risk factors in acute lymphoblastic leukemia patients.

Risk factors		GSTP1 in ALL(n=115)		OR	Lower	Upper	P value
		non mutant	mutant				
Family history	Yes	7	12	0.930	0.336	2.576	0.553
	NO	37	59				
Radiation	Yes	4	8	0.788	0.222	2.787	0.485
	NO	40	63				
Smoking	Yes	2	9	0.328	0.067	1.611	0.131
	NO	42	62				
Negative smoking	Yes	11	19	0.912	0.386	2.158	0.507
	NO	33	52				
Infection	Yes	25	38	1.143	0.536	2.436	0.440

DISCUSSION

Hazardous agents like toxins may lead to alterations inside different genes, which may increase susceptibility to cancer development, like ALL (Wogan *et al.,* 2004). Detoxification of xenobiotics or endogenous compounds introduced into the body will be done by important metabolizing enzymes like GSTs (Ketterer, 1988; Hengstler *et al.,* 1998). Hence alteration in that enzyme may alter their normal functional activity like in GSTP1 polymorphism (Zhong *et al.,* 2006; Lo and Ali-Osman, 2007).

To our knowledge, there are no reported genotype frequencies for GSTs polymorphism in acute lymphoblastic leukemicYemeni population. 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 GSTP1allelic frequency was 60% for the Ile/Val allele, 38.3% for wild type and only 1.7% for the Val/Val allele in ALL groups. While, the frequency of GSTP1allele was 42.1%, 38.3% and 2.9%, respectively in control population, it was found to be significantly elevated in patient with ALL (P value = 0.005). There are several studies agreed with our study about the role of the GSTP1IIe/105Val polymorphism in susceptibility to develop ALL in children. Other study by Krajinovic *et al.,* (2002) reported that there was an association between the GSTP1variants (alone or combined with other GSTs) and increased risk of developing childhood ALL. Also Canalle *et al.,* (2004)

observed that there was direct relation between the development of ALL and presence of carriers of the rare GSTP1105Val allele. On the other hand, Suneetha *et al.,* (2008) found no significant association between the risk factor and GSTP1gene for the development of ALL. Similar results were obtained by Guven *et al.,* (2015) and Ye and Song (2005).However, (Al-Eitan *et al.,* 2016) found that GSTP1 gene play role as risk factor for developing ALL in the of Arab children and there was a strong relationship between GSTP1 (Ile105Val) polymorphism genotypes and alleles within GSTP1 gene and ALL but no relationship between GSTM1 twofold invalid genotype and ALL (P=0.57) (Al-Eitan *et al.,* 2016).

In recent study we found strong association between ALL and GSTP1 variants (P value =0.005). The study was agree with several studies and the disagreement may relate to their low sample size or may be different genetic susceptibility of Yemeni patients vary between populations as well as different exposure to environmental factors such as toxins.

The combined effect among three genes (GSTT1 *null*, GSTM1 *null* and GSTP1 (Il105Val) there was highly significant difference between ALL patients and control 23(20%) in ALL and 8(5.7%) in controls {odds ratio (OR) 4.125, 95% confidence interval (CI) 1.768-9.626, P value =0.000}. These results agreed with combined analysis of GSTM1 and GSTP1 (IIe/Val)/(Val/Val) genotype done by (Suneetha *et al.,* 2008) he show significantly higher risk for ALL (OR=2.78: 95 CI=1.16-6.69).Similarly, higher risk was observed when combined GSTT1null, GSTM1null and GSTP1 variant genotype {P value =0.0364, odds ratio (OR) 6.20, 95% confidence interval (CI) 1.12-34.44} (Moulik *et al.,* 2014).

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