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Research Article

Influences of *Glutathione S-Transferase* Gene (*GSTT1*, *GSTM1*) Polymorphisms in Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Genetic variations in glutathione -S- transferase (*GST*), a gene that encodes carcinogenic metabolizing enzymes, could account for a proportion in leukemia. Authors investigated the genotyping polymorphism in glutathione –S-transferase mu1 (*GSTM1*) and theta 1 (*GSTT1*) in acute lymphoblastic leukemia (ALL). By using multiplex PCR, *GSTM1* and *GSTT1* polymorphisms were genotyped in 153 ALL adult patients and 90 healthy individuals served as control.
Results: Of the 153 ALL patients, 78.4% and 33.3% showed homozygous deletions in *GSTT1* and *GSTM1*, respectively. Significant association (P<0.0001) was reported between ALL patients (78.4%) as compared to controls (21.6%) regarding the *GSTT1* genotype polymorphism. Combination between the two candidate genotypes revealed significant level when deletions of the two genes were reported in ALL (relative risk =4.5, OR=61 and 95%CI= 6.7 – 599) as compared to control group.
Conclusion: Null *GSTT1* and *GSTM1* genotypes are associated with the risk of developing ALL.

Keywords: Glutathione S-transferase, ALL, polymorphism, multiplex PCR, *GSTT1*, *GSTM1*.

INTRODUCTION

DNA damage is the core stone in hematopoietic precursor cell for the development of leukemia[1]. This damage maybe mediated by reactive species generated by environmentally encountered carcinogens or endogenously as a result of oxidative metabolism[2]. Glutathione-s-transferases (GST) are super family of enzymes of phase II catalyzes the conjugation of reactive intermediates to soluble glutathione[3]. Among these are GSTM1 (μ) which detoxify carcinogenic polycyclic aromatic hydrocarbons such as benzo-(a) pyrene and GSTT1 (θ) which detoxify smaller hydrocarbons as ethylene oxide[3]. Deletion polymorphism in either *GSTM1* or *GSTT1* genotypes results in their aberrant catalytic activity, which is associated with greater sensitivity to toxic compounds, moreover it has been reported previously[4] that homozygous deleted genotypes of *dell/GSTM1* and *dell/GSTT1* is linked to enhanced genotoxicity and susceptibility to diseases such as leukemia[5]. Acute lymphoblastic leukemia (ALL) is a malignant heterogeneous disease seen in adults (Tang et al., 2014) in which the cells possess diverse phenotypes and variable response to chemotherapeutic regimens. Although previous studies have focused on assessing the susceptibility of GST polymorphisms with ALL[1,6,7] the association is still inconclusive. Thus in the current study authors aimed to investigates the association of *GSTM1* and *GSTT1* genotypes polymorphisms among adult Egyptian ALL patients with a focus on pre B- and T-phenotypes.

MATERIALS AND METHODS

Ethics, consent and permission

The current study was carried out after approval of the Ethical Committee from the National Research Centre and obtaining individual informed consent from all enrolled.

Enrolled individuals

Two-hundred fortythree individuals were included in the current study. One-hundred fiftythree ALL patients were obtained from newly diagnosed patients admitted to Department of Oncology, Faculty of Medicine, Alazhar University. Using French- American –British (FAB) diagnosis criteria, which was performed after conventional and surface-marker analysis the ALL patients were categorized accordingly into 12 (7.8%) with L1, 72 (47.1%) with L2 and 69 (45.1%) with L3 as reported in Table 1. Then a group (n=90) of healthy individuals were selected randomly to serve as control group.

Sample collection and DNA isolation

DNA was extracted from 3 ml of whole blood with EDTA using DNA purification kit (Promega Corporation) according to the manufacturer's instructions. Isolated DNA was stored at -20ºC till use.

Genotyping of GSTM1 and GSTT1polymorphisms

The *GSTM1* and *GSTT1* polymorphisms were detected using multiplex polymerase chain reaction (MPCR) in which *β-globin* gene was used as an internal control to avoid false negative readings as previously described[8]. Forwards and reverse primers for *GSTM1*: F5'-GAA CTC

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Table 1: Clinical characteristics and prognostic hematologic parameters in ALL patients.

Parameters	ALL (n=153)
Bone marrow blast cell count (%)[a]	73 (34-96)
Peripheral blast cell count (%)[a]	34 (9-82)
FAB classification, n (%)	
L1	12 (7.8)
L2	72 (47.1)
L3	69 (45.1)
Immunophenotype, n (%)	
T-ALL	30 (19.6)
B-ALL	123 (80.4)

[a]Median (range)
FAB: French–American–British

CCT GAA AAG CTA AAG C-3'; R5'-GTT GGG CTC AAA TAT ACG GTG G-3', *GSTT1*: F5'-TTC CTT ACT GGT CCT CAC ATC TC-3', R5'-TCA CCG GAT CAT GGC CAG CA-3', and for *β-globin*: 5′-CAACTTCATCCACGTTCACC-3′ and 5′-GAAGAGCCAAGGACAGGTAC-3′. PCR reaction was carried out using PCR master mix (Promega Cooperation, Cat no#7502) in a total volume of 25µl containing 10pmol/l of each primer, 2.5 mmol/l of MgCl2, 0.2 mmol/l of each dNTP, 1 U of *Taq* polymerase and 100 ng of genomic DNA. Amplification was carried out using Stratagene Mx3005P (Agilent Technologies, Germany) as follows: initial denaturation at 94 ℃ for 4 minutes, followed by 35 cycles at 94 ℃ for 1 minute, 54 ℃ for 45 seconds and 72 ℃ for 1 minute, and a final extension at 72 ℃ for 10 minutes. The amplified products were identified by electrophoresis in a 2% agarose gel and stained with 0.5 mg/ml ethidium bromide. Genotyping of *GSTM1* and *GSTT1*genes were considered absent (null) as their PCR products at 415 bp and 480bp, respectively were not detected as compared to the *β-globin* amplification at 268bp. Genotyping was considered "present" if one or two copies of the relevant gene were detected and classified as "null" if homozygous deletions was reported.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). The strength of the association in the polymorphism of *GSTM1* and *GSTT1* genotype with the allele between patients and controls was assessed by means of the odds ratio (OR) with 95% confidence intervals (CI) limits.

Analysis of variance (ANOVA) test was used to determine

the difference between the genotypes and continuous variables. Chi-square (X^2, two-sided) statistics were used to compare the association between the genotypes and alleles in relation to the cases and controls. *P* values <0.05 were considered as statistically significant.

RESULTS

The studied groups were of matched ages as their median [range] ages were as follows: 45 years [20 - 65 years] for controls individuals, and 45 years [23 - 65 years] for ALL patients, no significant difference was reported between controls and ALL regarding their ages. Similarly, gender status did not reach significant level as they were composed of 40, and 42 males and 50 and 46 females in controls and AML patients, respectively. Genotypic polymorphisms for *GSTM1* and *GSTT1* were detected in all enrolled individuals. Frequency for their expression did not reported significance between ALL patients and controls regarding their gender. The frequency of null *GSTM1* was non-significantly higher in control 44 (48.6%) as compared to ALL patients 51(33.3%), while null *GSTT1* was significantly higher in ALL patients 120 (78.4%) as compared to control group 24 (26.7%) with relative risk 0.72 which was higher than the relative risk of *GSTM1* (0.3%), as shown in Table (2). The combination between the genotyping polymorphisms for *GSTM1* and *GSTT1* (Table 3) revealed significantly higher frequency for null *GSTM1* or null *GSTT1* in ALL patients as compared to control individuals. The superior (4.55) relative risk was detected with dual null genotypes. The homozygous presence of the two genes was higher in control 32 (35.6%) individuals as compared to ALL patients 3 (2%). When authors assessed the correlation between ALL phenotypes and genotypic polymorphisms for *GSTM1* and *GSTT1* a significant correlation was reported between phenotypes and *GSTM1* (R=0.807, *P*=0.035) while the relation with *GSTT1* did not reach a significant level. In the current study ALL patients were categorized according to their phenotypes into 30 (19.6%) with T-ALL and 123 (80.4%) with pre B-ALL, accordingly the frequency for present/null candidate genes were investigated among the available immunophenotypes. As reported in (Table 4) patients with preB-ALL were having equal present /null *GSTT1* genotype polymorphisms [15 (50%)], for *GSTM1* the null genotype was higher as compared to the present

Table 2: Frequency of *GSTM1* and *GSTT1* genotypes in ALL and AML as compared to control individuals.

		ALL, n (%)			
Polymorphism	Control (n=90)	ALL Cases (n=153)	*P*-value	OR (95% CI)	RS
GSTT1					
Present	66 (73.3)	33 (21.6)	<0.0001	Ref.	0.72
Null	24 (26.7)	120 (78.4)		0.523 (0.9 – 1.07)	
GSTM1					
Present	46 (51.1)	102 (66.7)	NS	Ref.	0.3
Null	44 (48.9)	51 (33.3)		0.1 (0.03 – 0.25)	

Statistical significance using Pearson chi-square test. RS; relative risk, OR; odd ratio, CI; confidence intervals, NS; non-significance.

Table 3: *GSTM1* and *GSTT1* genotype polymorphism frequency in ALL as compared to control individuals.

	Control	ALL[a]	ALL *vs*. controls		
GST genotypes	n (%)	n (%)	RS	OR (95%CI)	*P*-value
GSTT1^{present}/*GSTM1* ^{present}	32 (35.6)	3 (2)		Ref.	
GSTT1^{null}/*GSTM1* ^{present}	34 (37.8)	33 (21.6)	1.15	10.35 (1.19 – 89.5)	0.014
GSTT1^{present}/*GSTM1* ^{null}	12 (13.3)	48 (31.4)	4.47	60 (6 – 566)	<0.0001
GSTT1^{null}/*GSTM1* ^{null}	12 (13.3)	69 (35.1)	4.55	61 (6.7 – 599)	<0.0001

Significance using Chi-square test(X^2) between controls with [a]ALL at X^2 =28.7, *P*-value <0.0001. Ref.; Reference, OR; Odd ratio, RS; relative risk.

Table 4: Association of genotypic polymorphisms and ALL phenotypes.

Genotypes polymorphisms	Pre-B-ALL (n=30)	T-ALL (n=123)	Statistics
GSTT1			
Present	15 (50%)	18 (14.6%)	X^2=5.94, *P*=0.015
Null	15 (50%)	105 (85.4%)	
GSTM1			
Present	6 (20%)	15 (12.2%)	X^2=0.995, *P*=0.318
Null	24 (80%)	26 (21.1%)	

genotype although no significant level was reached. Null *GSTT1* genotyping polymorphism in T-ALL [105 (85.4%)] was significantly higher than the present genotype for [18 (14.6%)], while for null *GSTM1*the frequency distribution was superior to the present *GSTM1* although it did not reach a significant level.

DISCUSSION

Among the prevalent malignancies in adult Egyptian patients is ALL[9] although progressing has been achieved in both diagnosis and treatment strategies[10]. Genetic polymorphisms in carcinogen-metabolizing genes such as GST are in the focus as they contribute to the susceptibility of ALL[11]. In the current study, authors investigate the association between *GST* (*GSTT1* and *GSTM1*) gene polymorphisms on the risk of adult ALL Egyptian individuals. The present data showed significant correlation between the null *GSTT1* and the susceptibility to ALL as the null *GSTT1* genotype polymorphism was significantly higher in 120 ALL out of 144 null *GSTT1* (83.3%) as compared to 24 controls out of 144 (16.7%), for null *GSTM1* genotype polymorphisms the association did not reaches a significant level although the frequency of genotype polymorphism was 51 ALL out of 95 with null *GSTM1* (53.7%) was higher as compared to 44 controls out of 95 (46.3%). These results were partially in agreement with previous studies, as some reported significant correlation with null *GSTM1* only[1,12] or no significant was reported although an increment in the frequency was detected in genotypes polymorphism in ALL as compared to controls[4]. 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[7]. Moreover, authors investigated the effect of the combined frequency for the candidate genotypic polymorphisms in all studied groups. As reported in (Table 3) significant difference was reported when either *GSTT1*or *GSTM1*null was considered as the frequency of ALL patients was higher than control individuals, moreover the odd ratio (OR= 61) and relative risk value (RS=4.55) reached the highest level when dull null genotypic polymorphisms were reported. These results could strength the correlation between GST genotypic polymorphisms and the etiology of lukomogenesis among ALL patients. In an attempt to investigate the association between genotypic polymorphism and ALL phenotypes, as reported in the current results null *GSTT1* genotypic polymorphism was significantly higher (*P*=0.015) in T-subtype (105 out of 120,87.5%) as compared to Pre B-subtype (15 out of 120, 12.5%). For null *GSTM1* genotypic polymorphism there was no significant differences between the two investigated subtypes although the null *GSTM1* was still higher in T-subtype (26 out of 50, 52%) as compared to Pre-B-subtype (24 out of 50, 48%). These results may indicate the linkages between the etiologies of T- subtype ALL and null *GSTT1* genotypic polymorphism, a prospective study is in progress with a larger number of ALL patients to investigate this association. In conclusion, genetic approaches to understand the linkage between the *GSTT1* and *GSTM1* genotypic polymorphism are delivering insights into the etiology of lukomogenesis and may in future direct the therapy strategies for ALL individuals with null *GSTT1* or null *GSTM1* genotypic polymorphisms who exposed to chemotherapeutic agents metabolized by GST enzyme.

AUTHOR'S CONTRIBUTION

MS and MSM carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. AK participated in the participated in the collection of samples and their diagnosis. MSM and AK participated in the design of the study. MS performed the statistical analysis. MS and MSM helped to draft the manuscript. All authors read and approved the final manuscript.

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