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Research Article

Role of Glutathione S-Transferase M1 and T1 Gene Polymorphisms in Adult Acute Myeloid Leukemia Susceptibility

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Abstract

Objectives: Authors aimed to investigate the association between *GSTT1* and *GSTM1* genetic polymorphism and susceptibility to acute myeloid leukemia (AML).

Materials and methods: Genotyping polymorphisms were studied in a total adult 178 individuals (88 newly diagnosed AML patients and 90 healthy controls) using multiplex polymerase chain reaction (PCR).

Results: Significant difference was reported between genotype polymorphism in AML as compared to control individuals. High risk incidence was detected when dual null of the candidate genotypes were combined ($p < 0.0001$).

Conclusion: Dual combination of the null *GSTT1* and *GSTM1* were superior to the susceptibility to leukemogenesis.

INTRODUCTION

The damage of DNA in hematopoietic precursor cells is directly linked to the risk of leukemias [1] this damage may be mediated by reactive oxygen species (ROS) generated by environmental or endogenous metabolites [2]. Human cells possess metabolic systems to eliminate toxic agents and several enzymes are responsible for the degradation of these xenobiotics, one systems being the glutathione S-transferase (GST, EC 2.5.1.18); group of enzymes coded by 16 genes, which conjugate/detoxify these carcinogens by conjugation with glutathione [3]. Moreover, *GSTs* genes are important in protecting against the ROS caused by the breakdown of the peroxidased lipids and they are capable of oxidizing DNA and generating damage [4]. Also they are involved in phase II metabolism, catalyzing the conjugation of soluble glutathione with reactive intermediates produced during the bio-activation of pro-carcinogens and detoxification of carcinogens [5], thus their polymorphisms may have a role in the susceptibility to leukemogenesis [1,6,7].

The GST isoenzymes expressed in human tissues comprise the alpha, mu, pi, theta, kappa, sigma, zeta and omega gene families [8], functional polymorphisms have been reported in five of them *GSTM1*, *GSTM3*, *GSTT1*, *GSTP1* and *GSTZ1* [9]. Many studies have been investigated polymorphism for genes encoding *GSTM1* (μ), *GSTT1* (θ) in many different populations, including those from Japan [10], Italy [11], Spain [12] and Brazil [13], however, their findings were in a conflict regarding the association between GST polymorphisms in AML susceptibility. The possible reasons for these discrepancies include differences in ethnicity, sample sizes and the age of the patients between the studies.

In the current study, authors aimed to investigate polymorphisms distribution of *GSTM1* (μ), *GSTT1* (θ) in a group of adults with acute myloid leukemia (AML) as compared with control individuals to investigate the possible association between different *GST* genes variants (*GSTT1*, and *GSTM1*) and incidence of AML.

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 individuals.

Enrolled individuals

A total of 88 newly diagnosed AML patients [14]. Diagnosis

was done according to standard morphological, cytochemical and immunophenotyping criteria following Leukemias and Lymphoma World Health Organization (WHO)-classification [15]. A group of 90 healthy individuals were enrolled in the study and their diagnosis was confirmed by their normal complete blood counts and no signs of hematological disorders were detected.

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 -20°C till use.

Genotyping of *GSTM1* and *GSTT1* polymorphisms

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 [16]. Forwards and reverse primers for *GSTM1*: F5'-GAA CTC 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 $MgCl_2$, 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°C for 4 minutes, followed by 35 cycles at 94°C for 1 minute, 54°C for 45 seconds and 72°C for 1 minute, and a final extension at 72°C 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 415bp 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

In the current study, authors assessed the frequency for the expression of *GSTM1* and *GSTT1* genotype polymorphism in a total of 178 individuals. They were divided clinically into 88 AML patients and 90 healthy volunteers. All enrolled individuals were of matched ages with median [range] ages were as follows: 45 years [20-65 years] for controls individuals, and 45 years [23-65 years] for AML patients, no significant difference was reported between controls and AML 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. Clinical characteristics and hematologic parameters for AML were reported in Table 1.

Table 2 showed genotype and allele distributions of the polymorphisms in the candidate genes for both control and AML patients. As reported for *GSTM1* genotype distribution significant difference was detected as the non-functional or the null *GSTM1* allele were reported at high incidence among AML patients as compared to control individuals. As for *GSTT1*, no significant difference was reported between both AML patients and control individuals although no significance was reported the *GSTT1* null genotype was more frequent in patients (54.5%) as compared to control (26.7%).

Given the biologically distinct morphological subtypes of AML the frequencies in null *GSTM1* polymorphism were non-significantly elevated among the M1, M2, M3, M4 and M5 subtypes [66.7%, OR, 0.523; 78.6%, OR, 0.285; 66.7%, OR, 0.523; 80%, OR, 0.261; 85.7%, OR, 0.174, respectively versus 51.1% for control]. For the frequencies of the *GSTT1* null genotype among the different AML subtypes, was elevated significantly only in M3 [66.7% versus 26.7% for control; OR, 4.3 at *P*=0.04] and M4 [80% versus 26.7% for control; OR, 8.5 at *P*=0.01] while it was non-significantly elevated among the M1, M2 and M3 subtypes [33.3%, OR, 0.727; 50%, OR, 0.364; 66.7%, OR, 0.523, respectively versus 26.7% for control].

Combination between the two investigated polymorphisms *GSTT1* and *GSTM1* in investigated groups, (Table 3). The relative risk level in AML was superior (relative risk=2.5) for those with $GSTT1^{null}/GSTM1^{null}$ followed by individuals with null allele of either of the genotypes, all reported to reach the significant level.

DISCUSSION

Although a progress in treatments for adult AML using aggressive treatments protocols, the 5-years disease free survival reported to be 20% and this may be referred to drug toxicity which is an important drawback for the success of treatment [17]. In the current study the genetic polymorphisms

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

Parameters	AML (n=88)
Bone marrow blast cell count (%)[a]	67 (20-98)
Peripheral blast cell count (%)[a]	42 (6-90)
FAB classification, n (%)	
M1	18 (20.5)
M2	28 (31.8)
M3	12 (13.6)
M4	10 (11.4)
M5	14 (19.5)
M6	6 (6.8)

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

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

		AML, n (%)			
Polymorphism	**Control (n=90)**	**AML (n=88)**	***P*-value[b]**	**OR (95%CI)[a]**	**RS[a]**
GSTT1 Present Null	 66 (73.3) 24 (26.7)	 40 (45.5) 48 (54.5)	NS	Ref. 0.52 (0.229 – 1.19)	0.71
GSTM1 Present Null	 46 (51.1) 44 (48.9)	 68 (77.3) 20 (33.7)	0.01	Ref. 0.307 (0.12 – 0.76)	0.58

[a] OR, 95%CI and RS used exact method, [a] Using Fisher's exact test
Abbreviations: OR: Odds Ratio 95%CI, 59% confidence intervals; RS: Relative Risk

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

GST genotypes	**Control n (%)**	**AML n (%)**	**AML vs. controls**		
			RS	**OR (95%CI)**	***P*-value**
$GSTT1^{present}/GSTM1^{present}$	32 (35.6)	6 (6.8)		1	
$GSTT1^{null}/GSTM1^{present}$	34 (37.8)	40 (45.5)	1.8	6.27 (1.55 – 25)	0.006
$GSTT1^{present}/GSTM1^{null}$	12 (13.3)	14 (15.9)	1.83	6.22 (1.2 – 32.7)	0.023
$GSTT1^{null}/GSTM1^{null}$	12 (13.3)	28 (31.8)	2.5	12.4 (2.6 – 59)	0.001

Significance using Chi-square test(X^2) between controls with AML at X^2 =12.4, *P*-value=0.006.
Abbreviations: Ref: Reference; OR: Odd Ratio; RS: Relative Risk.

for two candidate genes (*GSTT1* and *GSTM1*) were investigated in AML among Egyptian patients, were polymorphisms result in a lack of GSTs enzymatic activity causing the reduction of their detoxification role and may contribute to the susceptibility of leukemia [18]. *GSTM1* genotype was significantly different in AML patients as compared to the control group (p=0.01), while no significant difference was reported between *GSTT1* genotype between the two groups although null *GSTT1* was higher in AML groups as compared to the control individuals. These results may direct to the importance of these genotype polymorphism in the pathogenesis of AML because GST polymorphism indicates a lack in the functional protein either by reducing or increasing the enzyme metabolic activity thus increase the susceptibility to AML.

Combined null of the candidate genes reported to be at high risk of AML (Table 3). These trends in variability of null genotype polymorphism might aid to estimate the risk of individuals in developing cancer which may results from the imbalance between carcinogens and enzyme systems responsible for activation or detoxification of xenobiotics [19].

These results agreed with meta-analysis study [20] as it reported that dual null genotype *GSTT1* and *GSTM1* increased the risk of susceptibility to AML in both genotypes and this could be attributed to the fact that genetic polymorphisms could enhance genetic susceptibility to leukemogenesis [21]. In Caucasian population [22] the frequencies of the null genotype for *GSTM1* and *GSTT1* were in agreement to our findings as they ranged were 38% - 62% and 27% - 73%, respectively. In a recent report Karaca and his colleagues [23] among the Turkish population the frequencies of *GSTM1* (52%) were higher than our data while for *GSTT1* (23%) the frequency was nearly the same.

The differences among our results and previous studies could be attributed to i) the influence of *GSTT1* and *GSTM1* on genetic susceptibility of developing leukemia may vary between populations, ii) there are different patterns of carcinogen exposure involved in leukemogenesis due to specific gene-gene and gene-environment interactions, and iii) the variations in the number of the enrolled individuals in the genetic studies may have led to different outcomes [24], moreover, there were limitations to our study i) the number of individuals in the enrolled groups and ii) lack of inclusion for the risk predictors such as exposure to carcinogenesis as they affect the GST polymorphism.

In conclusion, our results have provided an evidence for association between *GSTT1* and *GSTM1* genotype polymorphisms and the increased risk for susceptibility of AML. The strength of our investigations includes comprehensive face-to-face interviews and access to DNA samples from enrolled individuals. Also large population-based studies are warranted to further investigate the impact of GST polymorphisms on AML susceptibility.

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