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Implications of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms for susceptibility to chronic lymphocytic leukemia

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Short communication

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ABSTRACT. Genetic polymorphisms involved in carcinogen metabolism contribute to leukemogenesis risk. Epidemiological studies have revealed genetic alterations in chronic lymphocytic leukemia (CLL), including chromosome alterations, noncoding RNA alterations, and genetic polymorphisms. The bioactivation and detoxification of chemical agents is mediated by xenobiotic metabolism enzymes, and genetic polymorphisms may explain interindividual differences in hematological cancer susceptibility. Glutathione S-transferase (GST) enzymes are capable of metabolizing xenobiotics into less toxic and more easily eliminated substances. Some studies have suggested involvement of *GST*s polymorphisms in leukemogenesis susceptibility. We investigated

possible genetic associations between *GSTM1*/*GSTT1* deletion and *GSTP1* rs1695 polymorphisms with CLL risk in a *central Brazilian population*. For *GSTM1*/*GSTT1* deletion polymorphism, genotyping was performed with multiplex real-time PCR (qPCR), and for *GSTP1* rs1695 polymorphism, PCR-RFLP was used. The *GSTM1* null genotype presented a trend towards CLL risk. However, *GSTT1* deletion polymorphism presented a protective effect for CLL (OR=0.26, p<0.005).The *GSTP1* rs1695 was not associated with disease susceptibility. Among confounding factors, male gender and age was associated with a 2.42-fold and 1.06-fold increased risk of CLL, respectively. In conclusion, among the polymorphisms that were evaluated, the *GSTM1* deletion polymorphism apparently helpsin the detoxification process and has a protective effect against CLL.

Key words: Chronic lymphocytic leukemia; Genetic polymorphisms; Glutathione S-transferase; Brazilian population

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder characterized by monoclonal B cell proliferation; it is more common in Western countries, accounting for 30% of cases of blood cancer in adults, especially affecting elderly male (Rodrigues et al., 2016). The etiopathology of this malignant disease is not yet completely elucidated; multiple factors could affect its occurrence (Hallek et al., 2019). Genetic epidemiologic studies have described genetic alterations in CLL, including chromosomal alterations, noncoding RNA changes, and single-nucleotide polymorphisms (SNPs) (Guven et al., 2015; Li et al., 2019).

Genetic polymorphisms involved in carcinogen metabolism contribute to leukemogenesis risk. (Guven et al., 2015; Nourizi et al., 2018). The polymorphisms in the genes responsible for encoding these enzymes may be associated with malignancies, since they encode enzymes with less activity and, consequently, less detoxification capacity of carcinogenic agents (Tsabouri et al., 2004). Some studies have suggested the involvement of *GST*s polymorphisms in leukemogenesis susceptibility (Yulle et al., 2002; Nourizi et al., 2018; Li et al., 2019).

The glutathione S-transferase (GSTs) family consists of several genes; *GSTM1*, *GSTT1*, and *GSTP1* are the most important ones (de Lima et al., 2018). These genes mediate the conjugation of reduced glutathione to electrophilic species, leading to the elimination of toxic compounds and thus favoring an antioxidant response (Pinheiro et al., 2013; de Lima et al., 2018; Nourizi et al., 2018). This action favors the process of cellular detoxification, contributing to the maintenance of genomic integrity and conferring protection of the organism against a range of products that could provoke oxidative stress. Additionally, changes in detoxification processes of toxic intermediates may be related to cell damage (Nourizi et al., 2018; Li et al., 2019).

GSTM1 and *GSTT1* deletion polymorphisms present homozygous deletions (*GSTM1*-null genotype and *GSTT1*-null genotype). In *GSTP1* rs1695 polymorphism, a

single nucleotide substitution causes the change of isoleucine to valine, decreasing enzyme activity. However, both polymorphisms may be able to influence enzymatic activity, impairing the detoxification capacity against carcinogenic agents. Thus these polymorphisms have been of considerable interest as candidate genes for CLL risk (Barros et al., 2021; de Lima et al., 2018; Li et al., 2019).

Few studies on *GSTs* polymorphisms and their association with CLL have been performed, especially in the Brazilian population. We investigated genetic association between *GSTM1/GSTT1* deletion and *GSTP1* rs1695 polymorphisms and the CLL risk in a Brazilian central population.

MATERIAL AND METHODS

In as case-control study, we investigated 74 patients with CLL in treatment at the Hematology Service, Clinical Hospital of the Faculty of Medicine, Federal University of Goiás, Goiânia-GO, Brazil. Our control group was composed of 106 individuals who were selected from the general population of the same region. Data on life habits, history of smoking and alcohol intake, as well as age and gender were obtained from medical records. This study was conducted under protocol number CAAE:21377413.7.0000.5078 according to the Research Ethics Committee of the Federal University of Goiás and in accordance with the Ethical Principles for Medical Research Involving Human Beings of the Declaration of the World Medical Association of Helsinki. All participants signed informed consent forms. Among the inclusion criteria, all 74 patients had their diagnosis confirmed by hematological service of the Clinical Hospital of the Federal University of Goiás. Absence of CLL diagnosis and impossibility of blood collection were considered exclusion criteria. We set the inclusion criteria according to The STrengthening the REporting of Genetic Association studies (STREGA) (Little et al., 2009) guidelines for improved reporting of genetic association studies.

Genomic DNA was extracted from peripheral blood samples with a commercial kit (Purilink Invitrogen®, USA). The *GST* deletion polymorphisms (*GSTM1* and *GSTT1*) were determined by multiplex Real-Time PCR (qPCR, SYBR Green®) followed by a melting curve analysis of the target sequence and reference gene (RH92600), as previously described (de Lima et al., 2018). Moreover, *GSTP1* rs1695 (Ile105Val) single nucleotide polymorphism (SNP) was determined through PCR - restriction fragment length polymorphism (PCR-RFLP). The PCR products were digested with 1.0 unit of restriction enzyme Alw26I, and results were observed in a 14% polyacrylamide gel electrophoresis, as previously described (de Lima et al., 2018).

Statistical analyzes were performed using RStudio software (v.1.0.153). Quantitative variables related to clinical and laboratory data were presented as mean and standard deviation. The categorical variables of genotypic distribution frequencies were summarized by absolute frequency (n) and relative frequency (%). Student's T-Test, Chi-Square Test (χ^2) or Fisher's Exact Test when necessary were used to compare demographic, biochemical, and clinical data of the participants (DN and control groups).

The odds ratio (OR) with the corresponding 95% confidence intervals (95%CI) and p-values were analyzed to estimate the risk of CLL by multiple logistic regression, assuming a genetic additive model adjusted for gender, age, smoking, and alcohol intake. For the analysis of the interaction of genotypes, multiple logistic regression was performed

considering triple interaction (*GSTT1*: *GSTM1*: *GSTP1*), double (*GSTT1*: *GSTM1*, *GSTT1*: *GSTP1*, *GSTM1*: *GSTP1*) genotypes were analyzed individually (*GSTT1*; *GSTM1*; *GSTP1*). For all tests, p-values under 0.05 were considered significant.

RESULTS

The demographic characteristics and lifestyle data (alcohol intake and smoking) of the CLL patients and controls are shown in Table 1.

Table 1. Demographic data of chronic lymphocytic leukemia patients and controls.

Variable	Case n (%)	Control n (%)	p
Age (years)	66.1 ±9.6	58.2 ±10.1	< 0.05*
Gender			
Female	32 (43.2)	71 (67.0)	< 0.05*
Male	42 (56.8)	35 (33.0)	
Alcohol intake			
Positive	17 (23.0)	29 (27.4)	0.51
Negative	57 (77.0)	77 (72.6)	
Smoking			
Positive	35 (47.3)	42 (39.6)	0.31
Negative	39 (52.7)	64 (60.4)	

Student t-test. *Significance between groups: P < 0.05.

In patients, the frequency of *GSTM1* null and *GSTT1* null genotypes were 32.4 and 35.1%, respectively. In this same group, 52.7% of patients with CLL were genotyped as heterozygous (Ile/Val) and 9.5% as mutant homozygous (Val/Val) for *GSTP1* rs1695 SNP. Meanwhile, in the control group, 46.2% and 12.3% were genotyped as *GSTM1* null and *GSTT1* null genotypes, respectively. Besides, for *GSTP1* rs1695 SNP, 53.8% and 9.4% of these healthy individuals were genotyped as heterozygous (Ile/Val) and mutant homozygous (Val/Val), respectively.

Table 2. Distribution of genotype frequencies of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in chronic lymphocytic leukemia patients and controls.

Genotype	Case n (%)	Control n (%)	χ^2	P	OR	P	CI (95%)
GSTM1							
Present	50 (67.6)	57 (53.8)	-	-	(1.0 Reference)	-	-
Null	24 (32.4)	49 (46.2)	3.44	0.09	1.79	0.06	0.92 – 3.50
Total	74 (100)	106 (100)					
GSTT1							
Present	48 (64.9)	93 (87.7)	-	-	(1.0 Reference)	-	-
Null	26 (35.1)	13 (12.3)	13.43	<0.005*	0.26	<0.005*	0.11 – 0.58
Total	74 (100)	106 (100)					
GSTP1							
Ile/Ile	28 (37.8)	39 (36.8)	-	-	(1.0 Reference)	-	-
Ile/Val	39 (52.7)	57 (53.8)	0	1	1.05	1	0.53-2.07
Val/Val	7 (9.5)	10 (9.4)	<0.01	1	1.03	1	0.31- 3.59
Total	74 (100)	106 (100)					

Chi-Square Test (χ^2). Multiple logistic regression and the allelic association was evaluated by Fisher's exact test. Abbreviations: OR= Odds Ratio; CI= 95% confidence interval. * Significance between groups: P < 0.05.

Analysis of the risk associated with the *GSTM1* deletion polymorphism revealed that the null genotype presented a trend of significance in predicting the occurrence of te CLL (OR=1.79; P = 0.06). On the other hand, *GSTT1* polymorphism indicated that a positive genotype could have a protective effect against carcinogenesis (OR=0.26; P < 0.005). However, the *GSTP1* rs1695 (Val/Val) genotype indicated no association with susceptibility in this population (Table 2).

The Hardy-Weinberg Equilibrium Test for the *GSTP1* rs1695 polymorphism indicated a greater amount of heterozygous individuals (Ile/Val) in both the case group (52.%) and the control group (53.8%). In addition, the mutant allele (Val) had a 36% frequency in both groups. The observed proportions were not significantly different from those expected in the case group (χ^2 = 1.59, P = 0.21), and in the control group (χ^2=2.80, P = 0.09) (Table 3).

Table 3. Genotypic and allele distribution of the *GSTP1* rs1695 polymorphism in chronic lymphocytic leukemia for Hardy-Weinberg Equilibrium test.

Genotype GSTP1	**Obs.**	**Exp.**	**χ^2 (D.F.)**	**P-value**
Case	**n (%)**			
ILE/ILE (Wild Type)	28 (37.84)	30.49		
ILE/VAL (Heterozygous)	39 (52.70)	34.02	1.59 (1)	0.21
VAL/VAL (Mutant)	07 (9.46)	9.49		
Total	74 (100)			
Alleles	**Frequency**			
ILE (Wild Type)	0.64			
VAL (Mutant)	0.36			
Control				
ILE/ILE (Wild Type)	39 (36.79)	42.98	2.80 (1)	0.09
ILE/VAL (Heterozygous)	57 (53.77)	49.03		
VAL/VAL (Mutant)	10 (9.44)	13.98		
Total	106 (100)			
Alleles	**Frequency**			
ILE (Wild Type)	0.64			
VAL (Mutant)	0.36			

Exact Test for Hardy-Weinberg equilibrium. Obs. – Observed; Exp. – Expected; DF – Degree of Freedom.

The multiple logistic regression allowed us to infer that there was no interaction between the three polymorphisms studied on the CLL susceptibility (OR=7.94; P = 0.15). This analysis also did not indicate a double interaction of *GSTM1*:*GSTP1* (OR=1.55; P = 0.47), *GSTT1*:*GSTP1* (OR=2.23; P = 0.25) and *GSTM1*: *GSTT1* (OR=4.76; P = 0.10). When the genotypes were evaluated by isolated form, there was no significant effect of the polymorphisms on the occurrence of CLL.

We observed a significant effect of *GSTT1* on CLL (OR=0.29; P = 0.004). However, the risk was not associated with increased susceptibility to the disease. In addition, it should be noted that among the confounding factors considered for modeling this regression, only gender and age were identified as risk factors indicating a 2.42-fold and a 1.06-fold risk increase of having CLL, respectively. On the other hand, the genotypic interaction among *GSTM1*, *GSTT1,* and *GSTP1* polymorphism for the lifestyles (smoking and alcohol intake) were not found for CLL risk (Table 4).

Table 4. Multiple logistic regression model for risk analysis in the chronic lymphocytic leukemia patients.

Adjustment Factors	OR	p
Gender	2.42	0.02*
Alcohol intake	0.58	0.20
Smoking	1.03	0.95
Age	1.06	0.0007*
GSTT1	0.29	0.004*
GSTM1	1.45	0.30
GSTP1	0.96	0.89
GSTT1:*GSTM1*	4.76	0.10
GSTT1:*GSTP1*	2.23	0.25
GSTM1:*GSTP1*	1.55	0.47
GSTT1:*GSTM1*:*GSTP1*	7.94	0.15

Multiple logistic regression showing adjusted odds ratio values (OR) for each tested variable. *Level of significance ($P < 0.05$).

DISCUSSION

It is known that cancer results from an imbalance between exposure to carcinogens and the effectiveness of the various enzymatic systems involved in the process of cellular detoxification (Reis et al., 2010; Li et al., 2019). The interindividual genetic variability in xenobiotic-metabolizing enzymes has been associated with the development of several types of cancer (Tsabouri et al., 2004; Carlsten et al., 2008; Reis et al., 2010; Li et al., 2019). Studies in *GST* polymorphisms have demonstrated that null genotypes result in reduced enzymatic activity (Tsabouri et al., 2004; Guven et al., 2015 Zehra et al., 2018). However, the influence of these polymorphisms in the hematological malignancy remains controversial.

For this reason, some genetic epidemiologic studies have investigated the influences of the *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms for the occurrence of some types of cancer, such as leukemias. Our data showed significant differences in gender and age. Thus, these findings are following data from the literature, demonstrating that elderly male is more predisposed to CLL (Tsabouri et al., 2004; Yuille et al., 2002; Rodrigues et al., 2016). Catovsky et al. (2014) suggested that differences between the gender for development and response to therapy in CLL may be associated with the effect of estrogen level. Moreover, a Brazilian study demonstrated that age is linked to CLL predisposition for *Methylenetetrahydrofolate reductase* (*MTHFR*) rs1801133 SNP (Reis et al., 2019).

Genetic association studies have been described the relationship between GST variants and risk of a hematologic malignancies, such as acute lymphoblastic and myeloid leukemia (Kassogue et al, 20015; Nasr et al., 2015). A Pakistani study demonstrated that *GSTM1* and *GSTT1* null genotypes do not influence acute lymphoblastic leukemia susceptibility among adult patients (Zehra et al., 2018). However, few studies evaluated the relationship between *GST* polymorphisms in CLL. In addition, we found that *GSTM1* null genotype presented a trend to CLL risk. In the meta-analysis (Li et al., 2019), the authors described that *GSTM1* deletion polymorphism influences the risk odds to CLL. On the other hand, a Canadian study suggested that *GSTM1* null genotype is involved childhood acute lymphoblastic leukemia risk (Krajinovic et al., 1999).

A study conducted by Kassogue et al. (2015) identified a slightly higher frequency of the *GSTM1* null genotype in the case group when compared to control. Despite this, was

no associated between this genotype and the occurrence of Chronic Myeloid Leukemia (CML). This information differs from our results. However, it is important to highlight that this same study also described an increased CML risk in male associated with the *GSTT1* null genotype. On the other hand, the combination of *GSTM1* null and *GSTT1* positive genotypes demonstrated limited CML risk. We identified that the *GSTT1* positive genotype could have a protective effect against carcinogenesis in CLL (OR=0.26; $p<0.005$). Kassogue et al. (2015) demonstrated that *GSTT1* positive genotype might be considered as protective against chronic myeloid leukemia (CML), similar our results. Some previous studies have reported an association of this polymorphism with other types of leukemia, for example, acute leukemia (Rollinson et al., 2000). Tang et al. (2013) described that *GSTT1* null genotype had a significant association with childhood acute leukemia risk, in the subgroup of Asian. Furthermore, these authors described also that *GSTM1* null genotype is involved with the susceptibility to childhood acute leukemia.

The GSTs influence the metabolism of carcinogens and are considering good candidates in the genetic studies for determining the leukemia risk. Their enzymes play role in the bioactivation and detoxification of chemical agents and, protect against reactive oxygen species. Thus, studies have been suggested the involvement of the GSTs polymorphism on the leukemogenesis susceptibility. (Nars et al., 2015).

Our findings by multiple logistic regression revealed that gender had a 2.42-fold increased CLL risk for the male (Table 3). This result is associated especially with the elderly male (Rodrigues et al., 2016; Hallek et al., 2019). It is suggested that this type of neoplasia presents a higher prevalence in male, being more common with advancing age, especially in Western countries (Reis et al., 2019).

In addition, we did not observe an interaction between the *GSTT1*, *GSTM1*, and *GSTP1* genotypes. The interaction analysis between *GSTM1* and *GSTT1* deletion polymorphisms also had a limited effect on the CLL risk. The study conducted for Yuille et al. (2002) evidenced trend to increasing risk for CLL for the mutant alleles from the GST family. Moreover, some studies suggested an association between the *GSTT1* null and *GSTM1* null variants and an increased risk of developing acute leukemia. Tsabouri et al. (2004) described that individuals with the *GSTM1* and *GSTT1* null genotypes may have enhanced susceptibility to CLL.

For lifestyle factors, such as smoking, our findings suggest that there is no association with the development of CLL. Similarly, a British study described no association between smoking and disease risk by *GST*s deletion polymorphisms in acute lymphoblastic leukemia (ALL) (Rollinson et al., 2000). Additionally, Kilfoy et al. (2009) described the relationship of *GST*s polymorphisms, smoking, and non-Hodgkin's lymphoma (NHL) no demonstrated an association between the GSTs variants and the pathology. Interestingly, Cerliani et al. (2016) showed no significant association between these polymorphisms and oncohematological diseases. These findings agree with our results, no association between smoking and the occurrence of CLL.

It is evident from these facts that the association between the GST variants and the susceptibility to CLL has not yet been fully elucidated. Furthermore, this is the first molecular study of GST polymorphisms in the Brazilian population for CLL. We suggest that the *GSTT1* positive genotype is has a protective effect against CLL. However, there is a high heterogeneity degree in the Brazilian population, suggesting that the distribution of these polymorphisms may vary as a consequence of ethnic factors. Consequently more

research on these polymorphisms need more investigations, especially in the Brazilian population.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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