



## POLYMORPHISM IN THE GLUTATHIONE S-TRANSFERASE THETA AND MU GENES AND SUSCEPTIBILITY TO LYMPHOID LEUKEMIA IN SUDANESE PATIENTS

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### ABSTRACT

**Background:** Glutathione S-transferase (GST) are a family of cytosolic enzymes known to catalyze the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress, by conjugation with glutathione. GSTs are able to modulate the induction of other enzymes and proteins important in cellular functions, such as DNA repair, and are therefore important in maintaining genomic integrity and, as a result, may play an important role in cancer susceptibility. **Aim:** This study aimed to study GSTT1 and GSTM1 null polymorphism in Sudanese patients with lymphoid leukemia (ALL and CLL) and correlate the polymorphism with patients age and gender. **Materials and Methods:** The study is a case control study, conducted in Khartoum state during the period from January to March 2017 among 20 patients with chronic lymphocytic leukemia among both gender at different ages and 20 patient with acute lymphocytic leukemia and 80 apparently healthy control subjects. The GSTT1 nul genotype was determined using polymerase chain reaction (PCR) method. **Results:** The GSTT1null polymorphism in patients with acute lymphoid leukemia was detected in 13 of patient (65%) 5of them were male and 8 were females, The GSTM1null polymorphism in patients with acute lymphoid leukemia was detected in 17 of patient (85%) 10of them were male and 7 were females. The GSTT1 null polymorphism was detected in 9 of control(18%) with insignificant(OR=8.5for ALL, 95% CI= (2.6- 27.2 P= 0.00). The GSTM1 null polymorphism was detected in 16 of control (53%) but the difference was statistically significant (OR=0.20for ALL, 95% CI=(0.049- 0.836 P= 0.021). The GSTT1 null polymorphism in patients with chronic lymphocytic leukemia was detected in 7 of patients(35%) 6 of them were male and 1 were females. The GSTM1 null polymorphism in patients with chronic lymphocytic leukemia was detected in 16of patients(80%) 10 of them were male and 6 were females. The GSTT1 null polymorphism was detected in 9 of control(18%) with insignificant (OR=2.4 for cLL, 95% CI=(0.763- 7.890 P= 0.126), The GSTM1 null polymorphism was detected in 16 of control(53%) with significant (OR=0.28 for cLL, 95% CI=(0.077- 1.058 P= 0.054). **Conclusion:** GSTT1 null genotype is a risk factor for ALL and there is statistically significant association between GSTT1 null polymorphism and ALL.

**KEYWORDS:** lymphoid leukemia (ALL and CLL), Polymorphism, GSTT1 and GSTM1.

### INTRODUCTION

**Acute lymphoblastic leukemia (ALL)**, is an acute form of leukemia, characterized by the overproduction and accumulation of cancerous, immature white blood cells, known as lymphoblasts.<sup>[1]</sup> In persons with ALL, lymphoblasts are overproduced in the bone marrow and continuously multiply, causing damage and death by inhibiting the production of normal cells (such as red and white blood cells and platelets) in the bone marrow and by spreading (infiltrating) to other organs. ALL is most common in childhood, with a peak incidence at 2–5 years. symptoms can include fever, increased risk of infection increased tendency to bleed (due to thrombocytopenia), and signs indicative of anemia, including pallor, fatigue, and headache.<sup>[1]</sup>

Internationally, ALL is more common in Caucasians than in Africans.<sup>[2]</sup> Acute" is defined by the World Health organization standards, in which greater than 20% of the cells in the bone marrow are blasts **chronic lymphoid leukemia (CLL)**, is the most common type of leukemia in adults.<sup>[1]</sup> CLL is a disease of the elderly;<sup>[4]</sup> however, in rare cases, it can occur in teenagers and occasionally in children. CLL is more common in men than women, with 63% of new cases occurring in men (UK, 2014).<sup>[3]</sup>

CLL affects B cell lymphocytes, which originate in the bone marrow, develop in the lymph nodes, and normally fight infection by producing antibodies. Most people are diagnosed without symptoms as the result of a routine blood test that shows a high white blood cell count

lymphocyte count is greater than 4000 cells per microliter ( $\mu\text{l}$ ) of blood, but can be much higher. As it advances, CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections. Chronic lymphocytic leukemia is defined as having less than 20% blasts in the bone marrow.

The GSTs<sup>[4]</sup> are a family of cytosolic enzymes known to catalyze the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress, by conjugation with glutathione. This conjugation reaction also facilitates excretion and thus constitutes a detoxification step. In addition to this role in phase II detoxification, GSTs are able to modulate the induction of other enzymes and proteins important in cellular functions, such as DNA repair, and are therefore important in maintaining genomic integrity and, as a result, may play an important role in cancer susceptibility.<sup>[5]</sup> It constitute multifunctional enzymes that are coded by at least eight distinct loci:  $\alpha$  (GSTA);  $\mu$  (GSTM);  $\theta$  (GSTT);  $\pi$  (GSTP);  $\sigma$  (GSTS);  $\kappa$  (GSTK);  $\omega$  (GSTO); and  $\zeta$  (GSTZ), each one composed of one or more homodimeric or heterodimeric isoforms. These enzymes are involved in the conjugation reactions between glutathione (GSH) and a variety of potentially toxic and carcinogenic compounds. Additionally, GSTs display peroxidase activity and this can protect against oxidative damage.<sup>[(6,7)]</sup> The deficiency in the activity of this enzyme can be derived from the inherited GSTs polymorphisms; GSTT1 (22q11.23), GSTM1 (1q13.3) and GSTP1 (11q13).<sup>[8]</sup> Three different polymorphisms have been described at the GSTM1 locus on chromosome 1p13.3.5. The most important polymorphism encodes for a partial

gene deletion in GSTM1 (GSTM1 null genotype) resulting in complete absence of GSTM1 enzyme activity. The 2 other polymorphisms do not lead to functional differences.<sup>[9]</sup>

The GSTM1 and GSTT1 loci have been mapped on chromosomes 1p13.3 and 22q11.2, respectively.

There is epidemiologic evidence that exposure to aliphatic hydrocarbons and chlorinated hydrocarbons plays a role in the etiology of CLL.<sup>3,5-8</sup> This, coupled with the proposed role of GSTs in the etiology of a number of common cancers<sup>[9]</sup> provides a strong rationale for evaluating *GSTM1* and *GSTT1* polymorphisms as risk factors for CLL.

## MATERIALS AND METHODS

### Study design, duration, and area

This is a case control study, conducted in Khartoum state, Sudan, during the period from January to March 2017.

### Study population and sample size

A total of 40 patients with CLL and ALL and 80 healthy volunteers as a control group were enrolled in this study.

### Sample collection and Molecular analysis

Venous blood sample was collected from each subject in ethylene diamine tetra acetic acid (EDTA) container and genomic DNA was extracted from whole blood samples by salting out method.<sup>[10]</sup> Samples will be stored at  $-30^{\circ}\text{C}$  until the analysis. GSTT1 and GSTM1 genes were genotyped using the multiplex PCR approach.

**Table 1: The primers sequence used for GSTT1 and GSTM1 amplification were.**

GSTM1	Forward primer 5'-GAACTC CCTGAAAAGCTAAAGC-3'
	Reverse primer 5'-GTTGGGCTCAAATATACGGTGG-3'
GSTT1	Forward primer 5'-TTCCTTACTGGTCCTCACATCTC-3'.
	Reverse primer 5'-TCACCGGATCATGGCCAGCA-3'.

PCR was performed on 4  $\mu\text{l}$  of DNA template, 1  $\mu\text{l}$  from each primer (Table 1) and 12  $\mu\text{l}$  of D.W in a total volume 20  $\mu\text{l}$  premix master mix (Intron. Korea).

Thermocycling conditions consisted of initial denaturation at  $95^{\circ}\text{C}$  for 5 mins, and then 35 cycles each consist of: denaturation at  $94^{\circ}\text{C}$  for 1min, annealing at  $60^{\circ}\text{C}$  for 1min, and extension at  $72^{\circ}\text{C}$  for 1 min, and followed by a final extension at  $72^{\circ}\text{C}$  for 5mins. PCR products were electrophoresed on 2% agarose gel containing ethidium bromide and visualized by gel documentation system. 100 bp DNA ladder was run with each batch of patients' samples. GSTT1 genotypes were determined by the presence and absence (null) of bands of 400 bp. GSTM1 genotypes were determined by the presence and absence (null) of bands of 219 bp.

### Ethical considerations

This study was approved by scientific research committee, faculty of medical laboratory sciences- Al Neelain University, and informed consent was taken from each participant before sample collection.

### Data collection and analysis

Patients' data was collected using structured interview questionnaire and analyzed by the statistical package for social sciences (SPSS). quantitative parameters were represented as mean  $\pm$  SD and compared according to GSTT1, GSTM1 genotypes using independent 2-sample T-test.

## RESULTS

This case control study includes 40 participants, 20 of them were Sudanese patients with acute lymphoid leukemia, 20 of them were Sudanese patient with chronic

lymphoid leukemia and 80 apparently healthy volunteers were included in the study as control group.

The GSTT1 null polymorphism in patients with acute lymphoid leukemia was detected in 13 of patient (65%) 5 of them were male and 8 were females, The GSTM1 null polymorphism in patients with acute lymphoid leukemia was detected in 17 of patient (85%) 10 of them were male and 7 were females. The GSTT1 null polymorphism was detected in 9 of control (18%) with insignificant (OR=8.5 for ALL, 95% CI= (2.6- 27.2 P= 0.00). The GSTM1 null polymorphism was detected in 16 of control (53%) but the difference was statistically significant (OR=0.20 for ALL, 95% CI=(0.049- 0.836 P= 0.021).

The GSTT1 null polymorphism in patients with chronic lymphocytic leukemia was detected in 7 of patients (35%) 6 of them were male and 1 were females. The GSTM1 null polymorphism in patients with chronic lymphocytic leukemia was detected in 16 of patients (80%) 10 of them were male and 6 were females. The GSTT1 null polymorphism was detected in 9 of control (18%) with insignificant (OR=2.4 for CLL, 95% CI=(0.763- 7.890 P= 0.126), The GSTM1 null polymorphism was detected in 16 of control (53%) with significant (OR=0.28 for CLL, 95% CI=(0.077-1.058 P= 0.054).

patients' ages with acute lymphoid leukemia were ranged from 4-62 years, 10 (50%) of patients were males and 10 (50%) of them were females. The patients' ages with chronic lymphocytic leukemia were ranged from 41-80 years, 12 (60%) of patients were males and 8 (40%) of them were females. There is no significant association between gender and the presence of the null polymorphism.

The frequency of GSTT1 Null polymorphism among patients with lymphoid leukemia was (65%) and (35%) in patients with CLL The frequency of GSTM1 Null polymorphism among patients with lymphoid leukemia was (85%) with significant difference 0.021 present, and (80%) in patients with CLL with significant difference 0.054 present.

The analysis show that the presence of GSTT1 polymorphism associated with 8.5 fold risk factor associated with ALL and 2.4 associated with CLL.

## DISCUSSION

Homozygotes for null alleles (deletion) of GSTM1 and GSTT1 have absent activity of the respective enzyme (Tang *et al.*, 2013).

The GSTs are involved in the metabolism of many environmental carcinogens, drugs and other xenobiotics. The polymorphisms result in a lack of enzymatic activity leading to a reduced detoxification role for GSTs. Thus the polymorphisms in the GST genes may be the factors

contributing to the differences in leukemia and susceptibility to other cancer types.

Several previous studies focused on the possible association between the polymorphism of GSTM1 and GSTT1 genes and the risk of childhood ALL and CLL development but the result are variable.<sup>[11]</sup>

This case control study was conducted to determine the frequency of GSTT1 and GSTM1 null genotype among Sudanese patients with childhood ALL and CLL to examine the association between GSTT1 and GSTM1 polymorphism and risk of developing childhood ALL and CLL.

-In this study we found that the GSTT1 null genotype was risk factors for ALL (OR = 8.5, 95% CI, *P*.value =0.00) and CLL (OR = 2.4, 95% CI, *P*.value =0.12) and this findings is in agreement with previous study done by Tang *et al* who performed a extensive meta-analysis on 26 published casecontrol studies which reported significant association between childhood acute lymphocytic leukemia risk, association was only found in a subgroup of Asian populations (OR = 1.94; 95% CI).

-Our finding is disagree with reports of Guvan *et al* who reported no significant association between GSTT1 and childhood ALL in a Turkish population (*P*.value =0.94).

-There is insignificant association between GSTT1 polymorphism and gender (*P*.value = 0.63) and this findings is in agreement with previous study done by Kassogue *et al* who reported that there were no significant association between GSTT1 and chronic myeloid leukemia in Morocco (*p*.value = 0.13).

-In this study we found that the GSTT1 null genotype was risk factors for CLL (OR = 2.4, 95% CI, *P*.value =0.12) and this findings is in agreement with previous study done by<sup>[12]</sup> to examine whether the GSTM1 and GSTT1 homozygous null genotypes altered the risk of CLL and they found a significantly increased incidence of the GSTM1 null genotype was found in the group of patients compared to the controls (74.07 versus 34.69%, *P*=0.0002).<sup>[10]</sup> The analysis show that the presence of GSTT1 polymorphism associated with 8.5 fold risk factor associated with ALL and 2.4 associated with CLL.

-In this study the analysis show that the presence of GSTT1 polymorphism associated with 8.5 fold risk factor associated with ALL and 2.4 associated with CLL and this is in agreement with study done to examine whether polymorphic variation in GSTs Confers susceptibility to chronic lymphocytic leukemia (CLL), GSTM1, GSTT1, and GSTP1 genotypes, They found that the frequency of both GSTM1 and GSTT1 null genotypes and the GSTP1-Ile allele was higher in cases than in controls and There was evidence of a trend in increasing risk with the number of putative "high-risk" alleles of the GST family carried (*P* =0.04).

The risk of CLL associated with possession of all 3 "high-risk" genotypes was increased 2.8-fold (OR = 2.8, 95% confidence interval: 1.1-6.9), the findings suggest that heritable GST status may influence the risk of developing CLL.<sup>[13]</sup>

### CONCLUSIONS

GSTT1 null genotype is a risk factor for ALL and there is statistically significant association between GSTT1 null polymorphism and ALL. There is no statistically significant association between GSTT1 and GSTM1 null polymorphism and gender.

### Recommendations

Another study should be conducted including information on the subtype of childhood ALL is demanded to clarify the relationship between the GSTT1 and GSTM1 polymorphisms and subtypes of childhood ALL.

Another study should be conducted with larger sample size to confirm the association of GSTT1 and GSTM1 null polymorphism with ALL and CLL.

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