**GENETIC POLYMORPHISM OF GSTT1 AND SUSCEPTIBILITY TO LYMPHOID LEUKEMIAS**

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**ABSTRACT**

**Background:** Glutathione S-transferase (GST) enzymes that play a key role in detoxification of activated carcinogens are shown to be one of the potential modifiers of individualized risk for several cancer types. **Objective:** This purpose of this study was to investigate the frequency of the GSTT1 null genotype in lymphoid leukemias (ALL and CLL) in Sudan. **Materials and Methods:** The study was conducted among 30 patients with chronic lymphocytic leukemia among both gender at different ages and 30 patient with acute lymphocytic leukemia and 60 apparently healthy control subjects. complete blood count were performed by an automated cell analyzer. The GSTT1 null genotype was determined using polymerase chain reaction (PCR) method. **Results:** The GSTT1 null polymorphism in patients with acute lymphoid leukemia was detected in 20 of patient (67%) 10 of them were male and 10 were females with insignificant, The GSTT1 null polymorphism was detected in 22 of control (23%), The GSTT1 null polymorphism in patients with chronic lymphocytic leukemia was detected in 16 of patients (53%). 13 of them male and 3 of them female, but the difference was statistically significant (OR=3.5 for both ALL and CLL, 95% CI= 1.8-4.2, P= 0.015) **Conclusion:** Our finding suggests that heritable GSTT1 status may influence the risk of developing CLL and ALL.

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

**INTRODUCTION**

There are basically four types of leukemia. The two types of leukemia derive from lymphocytes and are called acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Those are technically lymphomas, because they derive from lymphocytes. Because a lot of white blood cells will appear in the peripheral blood in a case of ALL, it is in fact also a leukemia. But some CLL patients actually have a normal white blood count and the "leukemia" cells will only reside in the lymph nodes for example. In this case the term leukemia is actually misplaced and a CLL should be termed lymphoma instead.

Note that ALL and CLL are not the only "lymphomas". There is a large variety of cancers that derive from lymphocytes or their precursors. The difference here is that those lymphoma cells will usually not appear in the peripheral blood which disqualifies them for the term "leukemia".

Chronic lymphocytic leukemia is the most common form of leukemia, accounting for around 30% of all cases. There is increasing evidence that predisposition to CLL involves both inherited and environmental factors.[1]

Several studies have found that there was association between glutathione s transferase gene polymorphisms and risk of hematological malignancies but in Sudan there is no published data regarding the role of GSTT1 polymorphism in development of CLL and ALL so the study will fill the gap.

Glutathione S-transferases (GSTs) are an important family of enzymes that catalyze the detoxification of a wide variety of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and reactive oxygen species.[2] They are involved in phase II detoxification, mediating the conjugation of reduced glutathione to electrophilic species that leads to the elimination of toxic compounds. GSTs have also been shown to play a role in kinase signaling and glutathionylation.[3] Therefore, GSTs play an important role in cellular protection from environmental exposures and oxidative stress and they are also implicated in cellular resistance to drugs.[4]

Inherited absence of alleles (null genotype) in GSTT1 genes result in lack of enzymatic activity.[5] The frequencies of GSTs polymorphic alleles, especially GSTT1 and GSTM1, have been reported in various
cancers.\textsuperscript{[6, 7, 8]} Several studies have been published on the relationship between GSTT1 and various types of cancers. In this study we evaluate the association of GSTT1 polymorphism and the susceptibility of polycythemia among Sudanese patient and To correlate the presence of this polymorphism with patients gender and age.

The design of study was a case control study. The study was conducted in Radio Isotope center Khartoum, in the period from May to August 2015. The study was conducted among 30 patients with chronic lymphocytic leukemia among both gender at different ages and 30 patients with acute lymphocytic leukemia and 60 apparently healthy control subjects. Three ml of venous blood was collected after disinfected with 70% alcohol using vacocontainer and dispensing into EDTA container.

Blood Samples were collected from individuals upon their agreement and the ethical approval well be obtained from the ethical committee of faculty of medical laboratory sciences, Al Neelain University.

Molecular analysis
DNA extraction
DNA was extracted by salting out method protocol; Samples were stored at -20°C until analysis.

Detection of GSTT1 polymorphism
Allele specific polymerase chain reaction (Technne, TC-412, UK) was used for detection of the polymorphic deletion of the GSTT1. The following pair of primers was used in the genotyping analysis: Sense primer: 5'-TTCCCTACTGTTGCTTACATCCT-3'. Antisense primer: 5'-TCACGGATCATGGCCAGCA-3'. PCR was carried out in a total volume of 20 μl. It consist of 4 μl of genomic DNA, 1 μl from each primer, 5 μl of “5X FIREPol” ready to load master mix (SOLIS BIODYNE, TARTU-ESTONIA) and 14 μl distilled water. PCR was initiated by denaturation step at 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C for 45 seconds, annealing temperatures ranged between 63°C for 1 minutes and 55°C for 30 second, extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. After amplification, 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 489 bp.

The presence of the GSTT1-active genotype was detected by the band at 489bp, since the assay does not distinguish heterozygous or homozygous wild-type Beta-globulin was also tested for because the absence of GSTT1 amplification product in the presence of the beta globulin PCR product indicates a GSTT1-null genotype. The beta-globulin primers used were F - 5'-GAA GAG CCA AGG ACA GGT AC-3’ and the product 489-bp.

Statistical analysis
The statistical analysis will be done by the SPSS. Data was recorded in or – standard deviation. Achi-square ($x^2$) test was used to compare genotype .p-value smaller than 0.05 were accepted as being statistically significant.

RESULTS
This case control study includes 90 participants, 30 of them were Sudanese patients with acute lymphoid leukemia, 30 of them were Sudanese patient with chronic myloid leukemia and 30 apparently healthy volunteers were included in the study as control group.

The GSTT1 null polymorphism was detected in 22 of control (23%) and GSTT1 normal was detected in 39 of control subjects (78%), 22(73%) of controls were males and 8 (26%) were females.

The patients’ ages with acute lymphoid leukemia were ranged from 4-75 years, 14 (47%) of patients were males and 16 (53%) of them were females with (value 0.3), The GSTT1 null polymorphism was detected in 20 of patient (67%) 10 of them were male and 10 were females with insignificant and GSTT1 normal genotype was detected in 10 of patients (33%) 4 male and 6 female with (p value 0). Mean of patients’ white blood cell and blasts were 43 (p value 0.5) and 46 respectively and standard deviation 38.5, 21.9 respectively. Mean of patients’ lymphocyte, neutrophils, monocytes, eosinophils were 45 (p value 0.3), 10, 0.8 and 0.5 respectively with standard deviation 22.6, 1, 2, 1.2 respectively. Mean of patients’ red blood cell was 3.4 with (p value 0.1) and stander deviation 0.9. Mean of patients’ MCV, MCH, MCHC and PCV were 7.6 (p value 0.5), 85.8 (p value 0.4), 27.5 (p value 0.8) and 31.6 (p value 0.4) respectively, with stander deviation 2.5, 7.2, 3.1, 2.9 respectively. Mean of patients’ platelet 79 with (p value 0.4) and stander deviation 12.

The patients’ ages with chronic lymphocytic leukemia were ranged from 32- 80 years, 22 (73%) of patients were males and 8 (26%) of them were females. The GSTT1 null polymorphism was detected in 16 of patients (53%). 13 of them male and 3 of them female and GSTT1 normal was detected in 14 of patient (47%). 9 of them male and 5 of them female with (p value 0.004). Mean of patients’ white blood cell and blasts were 88 (p value 0.5) and 0 (p value 0) respectively with standard deviation 10.4 and 0 respectively. Mean of patients’ lymphocyte, neutrophils, monocytes, eosinophils were 78, 16.5, 4.2 and 1.1 respectively with standard deviation 10, 3, 3, 1 respectively. Mean of patients’ red blood cell was 3.5 (p value 0.5) with standard deviation 3.4. Mean of patients’ MCV, MCH, MCHC, PCV were 10.2 (p value 0.4), 92 (p value 0.6), 29 (p value 0.6) and 31 (p value 0.6) respectively with standard deviation 0.
respectively. Mean of patients’ platelet 172 (p value 0.3) with standard deviation 66.

But the difference was not statistically significant (OR=3.5 for both ALL and CLL, 95% CI= 1.8-4.2, P= 0.015). Therefore GSTT null genotype may be a risk factor for both acute myeloid leukemia and chronic lymphocytic leukemia with (p value 0.2 and 0004 respectively).

There is no significant association between gender and the presence of the mutation (P.Value: 0.2 and 0.004 respectively).

The study also observe that GSTT1 null genotype was not related to red cell parameters a in both acute lymphoid leukemia and chronic lymphocytic leukemia (Table 1).

The frequency of GSTT1 Null polymorphism among patient with lymphoid leukemia were as that patient with CLL presented with 16% and those with ALL 20% with significant difference 0.015 present.

The analysis show that the presence of GSTT1 polymorphism associated with 3.5 fold risk factor associated with CLL and ALL.

**DISCUSSION AND CONCLUSION**

Homozygotes for the null alleles (deletion) of GSTM1 and GSTT1 lack activity of the respective enzymes,[8] this decrease the reactivity of electrophilic substrates, which may affect the functions within cellular macromolecules, such as nucleonic acid, lipid and protein. So, the genetically determined differences in metabolism, related to GST enzymes, have been reported to be associated with various cancer susceptibilities.[9] Positive associations were found in certain types of cancers while not found in others.

In our study we conclude that the GSTT1 null genotype was found to be significance association for increasing lymphoid leukemia risk (OR=3.5 for both ALL and CLL, 95% CI= 1.8-4.2, P= 0.015) and without relation in ages, gender and other haematological finding. This finding is in agreement with other studies, in which other cancers were studied. A study of Martin et al in 2002 (1) on patients with chronic lymphocytic leukemia showed that heritable GST status may influence the risk of developing CLL. other study in 2001 on children from north Portugal with acute lymphocytic leukemia showed to be a significant predictor of ALL risk. In contrast also many studies showed negative results in the association between GSTT1 null genotype and various types of diseases and cancers. A study in 2009 on Turkish patient showed no significance association of GSTT1 with cervical cancer.

All these xenobiotic-metabolizing enzymes and other related enzymes should be studied in different populations and in larger numbers. Also, other environmental factors such as smoking, drug treatments and exposure to radiation as a risk factor for increasing susceptibility to lymphoid leukemia should also be studied this can help in disease prevention. The limitation of our study was the small sample size so; we recommended that further study with increased sample size should be conducted in the future.

**REFERENCES**


6. Data from KEGG and Bio Carta [BIOCARTA terms] via CGAP.


