

Glutathione S-transferase M1null Genotype among Sudanese patients with Chronic Lymphoid Leukaemia attended at RICK(Radiation Isotope Center-Khartoum)

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Abstract

Background: Chronic lymphoid leukemia (CLL) is the most common form of leukemia, which affects the lymphoid series. The etiology of leukaemia is not fully understood, it thought to result from a combination of factors that include genetic mutations in cells and allow mutated cells to proliferate.

Glutathione sulfur transferase (GSTs) is a group of enzymes involved in the detoxification process of carcinogens and other substances. The genes encoding is enzymes M1 and T1 are polymorphic in humans and the phenotypic absence of enzyme activity (null genotype) may have an effect on the risk of several cancers.

This study aim to determine the association between Glutathione S-transferes gene polymorphism (M1null) and CLL in a group of Sudanese patient. There was Several studies have found association between glutathione S transferase gene polymorphisms (M1null) and CLL but in sudan there is no published data so the study will fill the gap.

Materials and methods: A total of 50 patients diagnosed with CLL attending to the Radiation and Isotope Center of Khartoum (RICK) Sudan, and 50 healthy volunteers as control group were enrolled in this study. For molecular analysis genomic DNA was extracted from participant's

EDTA anticoagulated blood samples by salting out method and analyzed by allele specific PCR for determination of GST(M1null).

Results: A total of 50 patients attended the Radio Isotope Center of khartoum, diagnosed with(CLL) their ages ranged between 40-78 years (mean \pm SD:63 \pm 8), They were correlate with 50 healty volunteers as control group their ages ranged between 42-75years (mean \pm SD:60 \pm 8).15 patients (30%) suffering from massive splenomegaly, 6 patients (12%) suffering from hepatomegaly.The frequency of GST(M1) comprised 26 (52%)among patients and GST(M1null) comprised 24(48%) of patients, In addition, the percentage of GSTM1 null genotype in CLL patients was significantly higher than in controls.

Conclusion: Our findings does not suggest that heritable GSTM1null may influence the risk of developing CLL,furthermore other study must be done with large sample size to support this study.

Keywords :CLL,GST, M1null

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Introduction

CLL is a disease state characterized by the proliferation of abnormal, developmentally immature B cells⁽¹⁾.CLL is a disease of adults, but, in rare cases, it can occur in teenagers and occasionally

in children (inherited). Most (>75%) people newly diagnosed with CLL are over the age of 50 years, and the majority are men. CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections⁽²⁾.

Glutathione S-transferases (GSTs), a superfamily of phase II metabolic enzymes, catalyze mainly the conjugation of glutathione or glucuronide with reactive electrophiles and thus detoxify procarcinogens and carcinogens⁽³⁾. These enzymes are widely expressed in mammalian tissues and have broad substrate specificity⁽⁴⁾. Two widespread genetic polymorphisms that involve deletions in the GSTT1 and GSTM1 genes, namely del{GSTT1} and del{GSTM1}, have been reported to lead to abrogation of enzyme activity⁽⁵⁾. The frequencies of GSTs polymorphic alleles, especially GSTT1 and GSTM1, have been reported in various cancers⁽³⁾⁽⁶⁾⁽⁷⁾.

Glutathione S-transferases (GSTs), previously known as ligandins, comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification. The GST family consists of three superfamilies: the cytosolic, mitochondrial, and microsomal—also known as MAPEG—proteins.⁽⁸⁾⁽⁹⁾⁽¹⁰⁾ Members of the GST superfamily are extremely diverse in amino acid sequence, and a large fraction of the sequences deposited in public databases are of unknown function⁽¹¹⁾. The Enzyme Function Initiative (EFI) is using GSTs as a model superfamily to identify new GST functions. GSTs can constitute up to 10% of cytosolic protein in some mammalian organs⁽¹²⁾⁽¹³⁾. GSTs catalyze the conjugation of GSH — via a sulfhydryl group — to electrophilic centers on a wide variety of substrates in order to make the compounds more water soluble⁽¹⁴⁾⁽¹⁵⁾. This activity detoxifies endogenous compounds such as peroxidized lipids and enables the breakdown of xenobiotics. GSTs may also

bind toxins and function as transport proteins, which gave rise to the early term for GSTs, *ligandin*⁽¹⁶⁾⁽¹⁷⁾.

Materials and Methods

Patients and Samples

A total of 50 Sudanese patients with CLL attended to Radiation and Isotopes center of Khartoum (RICK) newly diagnosed by complete blood count(CBC) and bone marrow during the period from May to September 2014 were enrolled in this study, their age over 40 years as well as the 50 apparently healthy volunteers were recruited to participate in this study their age more than 42 years. 2.5 ml of venous blood was collected from each subject in ethylene diamine tetraacetic acid (E.D.T.A) container for molecular analysis, and informed consent was taken from each participant.

DNA Extraction

DNA was extracted from (E.D.T.A) anticoagulated blood sample using salting out method.

Glutathione S-transferase gene polymorphism analysis

Glutathione S-transferase gene was detected using Allele-specific polymerase chain reaction (TC 412 -UK). Two micro liters (2µl) of DNA were amplified in a total volume of 22 µL containing one µl of each two forward primer F - 5'-GAA GAG CCA AGG ACA GGT AC-3'. and F - 5'-GAACTCCCTGAAAAGCTAAAGC-3'.and two reverse primer R- 5'-CAA CTT CAT CCA CGT TCA CC-3'. and R-5'-GTTGGGCTCAAATATACGGTGG-3', four µl Matser mix (GoTaq® Green Master Mix, Promega, USA) and 12µl sterile distilled water. The cycling conditions include initial denaturation at 94°C for 3 minutes; 35 cycles of 94°C for 1 minute(denaturation), 59°C for 1 minute(anealing), and 72°C for 1.5 minute(exetention) and;

final extension at 72°C for 10 minutes. Four µl of the PCR product (ready to load) was electrophoresed on 2% Agarose gel, stained with ethidium bromide and then demonstrated by gel documentation system. Gel electrophoresis showed presence of 268bp band only as GSTM1null and 219 bp with 268 bands as GSTM1.

Statistical analysis

Data of this study was analyzed by Statistical Package for Social Science (SPSS). Correlation between Glutathione S-transferase gene and qualitative variables were tested by cross-tabulation and chi-square test.

Results

A total of 50 patients diagnosed with chronic lymphocytic leukemia attended to the Radiation and Isotope Center of Khartoum (RICK) Sudan, their ages ranged between 40-78 years (mean ± SD: 63 ± 8), 34 (68%) of them were males and 16 (32%) were females, compared to 50 volunteers as control group their ages ranged between 42- 75 years, 35 (70%) of them were males and 15 (30%) of them were females. The frequency of GST(M1) comprised 26 (52%) among patients and GST(M1null) comprised 24 (48%) of patients was shown in figure 1. The statistical analysis showed that there was a significant difference in the frequency of Glutathione S-transferase gene between patients and control group. In addition, the percentage of GSTM1 null genotype in CLL patients was significantly higher than in controls. The result showed that there was insignificant difference between patient phenotype and TWBCS count p.value (0.418) and also between absolute lymphocyte count with patient phenotype P.value (0.432), while the results showed that there was no significant difference between patient

phenotype and Hemoglobin(Hb), p.value(0.463), Red Blood Cells (RBCs) p.value(0.458), Hematocrit(HCT%) p.value(0.610) and platelets (Plts) p.value(0.339) .there was 15 patients (30%) suffering from massive splenomegaly and six patients (12%) suffering from hepatomegaly. The result also showed that there was no significant different between patient phenotype and splenomegally p.value(0.574) , loss of weight p.value(0.788) and lumphadenopathy p.value(0.306).The figure 2 showed the mean of the haematological parameter among patient phenotype, so the mean of TWBCs among patients phenotype is (M171.12), (M1null:72.36), Hb(M1:9.78), (M1null:9.95) and platelets(M1:97.145), (M1null:93.972). Other wise there was insignificant difference between patients phenotype with patients age, gender and duration of the disease. the result showed that there was no correlation between patient phenotype when compared with patient lymphadenopathy, loss of weight,fatigue and fever.

Table.1: The P.Value of different variables

Variable	P.Value
Pt genotype\pt TWBCs	0.418
Pt genotype\pt absolute lymphocyte	0.432
Pt genotype\ptRBCs	0.458
Pt genotype\ptHB	0.463
Pt genotype\pt ptHCT%	0.610
Pt genotype\pt PLts	0.339
Pt genotype\pt splenomegaly	0.574
Pt genotype\pt loss of weight	0.788
Pt genotype\ lymphadenopathy	0.306

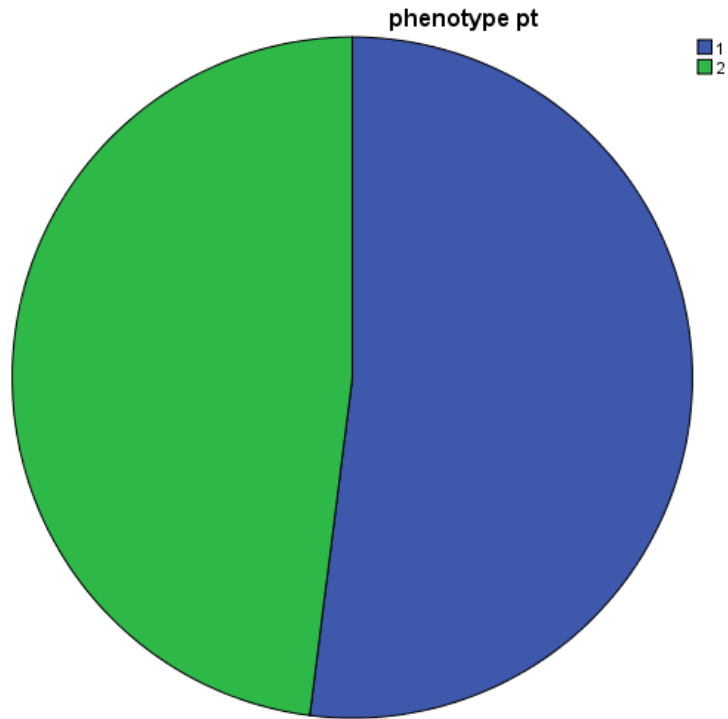


Figure.1: The frequency of GST among patient{1=M1(52%),2=M1null(48%)}

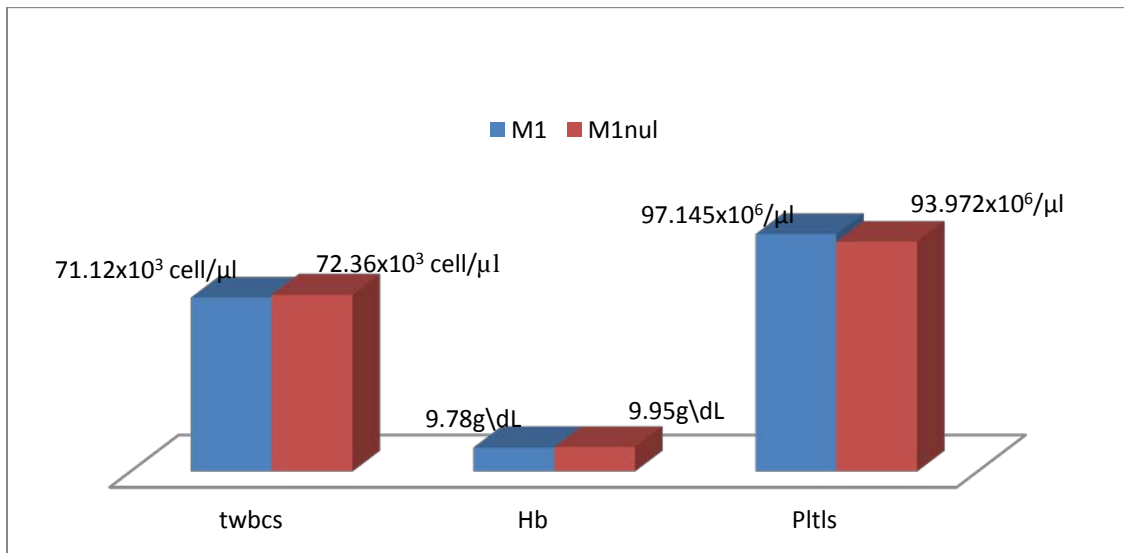


Figure.2: Mean of TWBCs,HB and platelets among patient phenotype.

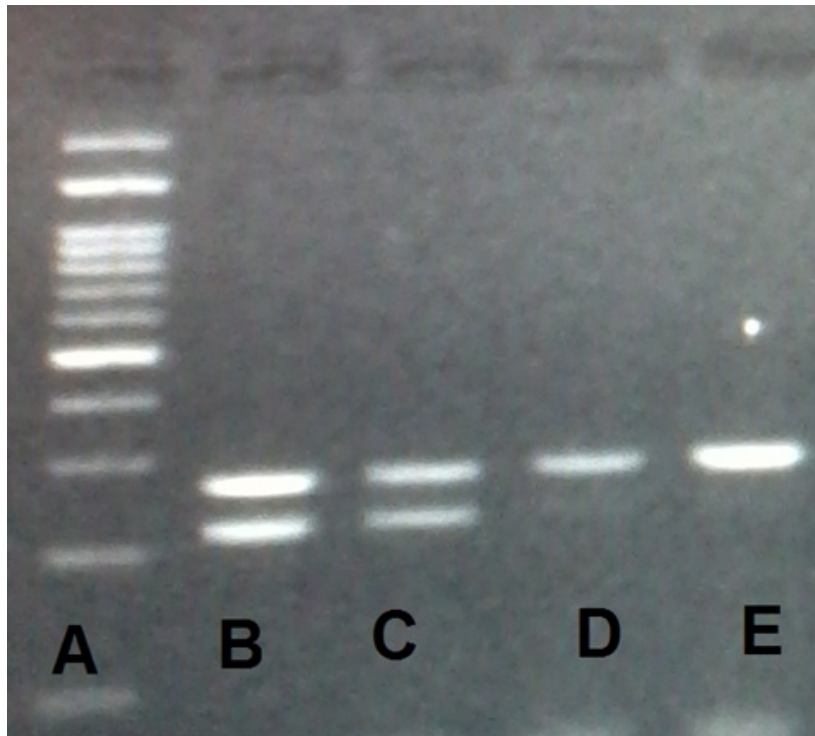


Figure 3: Shows GSTM1 and M1null genotypes which were performed using allele specific PCR(electrophoresis in 2% agarose gel). Genotype patterns: A:ladder 100bp . B:Control positive (219\268) bp,C : GSTM1(219\268)Sample No (6) ,D: GSTM1null(268)bp Sample No(7), E:Control negative(268)bp.

Discussion

The genetically determined differences in metabolism, related to GST enzymes, have been reported to be associated with various cancer susceptibilities. The GSTM1 null genotype causes accumulation of DNA adducts that may result in mutations in oncogenes or tumor suppressor

genes that may increase susceptibility to cancer development. Positive associations were found in certain populations for certain cancers while not confirmed in others. ⁽¹⁸⁾.

This study was performed to investigate the association between the Glutathione S-transferase gene (M1null) and (CLL) in Sudanese patients. We found that there is no association between GST(M1null) and CLL risk (OR = 0.664, 95% CI = 0.299–1.427, P = 0.210). This finding disagrees with study done by Sophia Tsabouri *et al* ⁽¹⁹⁾ which showed that the individuals with GST's null genotypes may have an increased susceptibility to develop CLL. The present study also disagrees with study done by Martin Yuille *et al* ⁽²⁰⁾ which showed that the heritable GST status may influence the risk of developing CLL. A study in Eastern India has showed a significant increase for GSTM1null genotype in AML group compared to normal group with (OR = 3.2595% CI = 1.9–5.58, p = 0.001) ⁽²¹⁾. Another study in China on leukemia patients showed a significance association with the GSTM1null genotype and development of Acute non Lymphocytic Leukemia (ANLL) (OR = 1.8, 95% CI = 1.11– 2.97) ⁽²²⁾. In another study, we showed that individuals with GSTM1 null genotype had an increased susceptibility to develop myelodysplastic syndrome. ⁽²³⁾

Although, the number of CLL patients, associated with GST genotypes is limited and further studies are welcomed to address our findings, by using large sample size.

Conclusion

Our findings does not suggest that heritable GSTM1null may influence the risk of developing CLL.

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