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GSTM1 POLYMORPHISM IN SUDANESE PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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ABSTRACT
Background: Glutathione S-transferase 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 GSTM1 null genotype in chronic lymphocytic leukemia patients in Sudan. **Material and methods**: Fifty chronic lymphocytic leukemia patients and fifty controls were evaluated to determine the frequency of gstm1 null genotype. The GSTM1 null genotype was determine using polymerase chain reaction (PCR) method. **Results**: The GSTM1 null polymorphism was detected in 38% of cases and 36% of control subjects (table1) but the difference was statistically significant (or=2.9 95% C l = 1.28 -6.5, p=0.01) therefore GSTM1 null genotype may be a risk factor for CLL. (P. value =0.01). **Conclusion:** In summary we concluded that GSTM1 null polymorphism is a risk factor for CLL among Sudanese patients.

KEYWORDS: Glutathione S-transferase among Sudanese patients.

INTRODUCTION
B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia, accounting for around 30%of all cases (Miller et al). The incidence rate of CLL increases logarithmically from the age of 35 years with a median age of diagnosis at 65 years (linet et al 1998). Is a type of slow growing leukemia that affects developing *B-lymphocytes* (also known as B-cells).These cells are specialized white blood cells. Under normal conditions they produce immunoglobulins (also called antibodies) that help protect our bodies against infection and disease. In people with CLL, lymphocytes undergo a malignant (cancerous) change and become leukemic cells. They live longer than they should and accumulate in the bone marrow, blood stream, lymph nodes (glands), spleen, liver and other parts of the body. Over time, an excess number of lymphocytes crowd the bone marrow, and interfere with normal blood cell production. The bone marrow produces inadequate numbers of red cells, normal white blood cells and platelets. This leads to some people with CLL being more susceptible to anemia, recurrent infections and bruising and bleeding easily. Circulating red blood cells and platelets can also be damaged by abnormal proteins made by the leukemic cells (3).

Glutathione S-transferases (GSTs) are a family of cytosolic enzymes involved in the detoxification of various exogenous as well as endogenous reactive species(ketterer et al 1988).GSTs function as dimmers by catalyzing the conjugation of mutagenic electrophilic substrates to glutathione. Polymorphisms in GST gene family casue a decrease or loss in activity of the corresponding enzymes and lead to the accumulation of intracellular genotoxic metabolites, which resulted in impairment of the cancer prevention mechanisms (Guengerich et al 1995). Three members of the GST enzymes; GSTM1, GSTP1, and GSTT1 catalyze the reactions with common carcinogens Inherited absence of two alleles (null genotype) in GSTM1 and GSTT1 genes result in lack of enzymatic activity (turesky et al 2011). 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 (Bolufer et al 2007). Inherited absence of alleles (null genotype) in GSTT1 genes result in lack of enzymatic activity (Srivastava et al 2005). The frequencies of GSTs polymorphic alleles, especially GSTT1 and GSTM1, have been reported in various cancers (taspinar et al 2008). in this study we evaluate the association of GSTM1polymorphism and the suscepility of chronic lymphocytic leukemia (CLL)among Sudanese patient and to correlate the presence of this (CLL) with patients age.

MATERIALS AND METHODS
This study is a case-control study, conducted in Khartoum state, Sudan, in the period from 2016. It is included 50 patients with chronic lymphocytic leukemia and 50 healthy volunteers as control group. Blood

samples were collected from all subjects in ethylene demine tetra acetic acid (EDTA) for measurement of red cell parameters using automated hematology analyzer "Sysmex KX-2IN, Japan". The control group consisted of healthy volunteers without a medical history of cancer or other diseases. This study was approved by ethical committee of the faculty of medical laboratory sciences, Al neelain University, and informed consent was obtained from each participant before sample collection.

Molecular analysis

DNA extraction: Genomic DNA was extracted by using salting out method. DNA samples were stored at -80°C until analysis.

Detection of GSTM1 polymorphism

Allele specific polymerase chain reaction was used for detection of the chronic lymphocytic luekemia deletion of the GSTM1. used the following forward (F) and reverse (R) primers:

F - 5'-GAACTCCCTGAAAAGCTAAAGC-3'.

R-5'-GTTGGGCTCAAATATACGGTGG-3'.

The presence of the GSTM1-active genotype was detected by the band at 215 bp, since the assay does not distinguish heterozygous or homozygous wild-type Beta-globulin was also tested for because the absence of GSTM1 amplification product in the presence of the beta globulin PCR product indicates a GSTM1-null genotype. The beta-globulin primers used were

F - 5′-GAA GAG CCA AGG ACA GGT AC-3′, R- 5′-CAA CTT CAT CCA CGT TCA CC-3′ and the product 268-bp.

PCR was carried out in a total volume of 20 µl. It consist of 2 µl of genomic DNA, 1 µl from each primer, 4 µl of "5X FIREPoL" ready to load master mix (SOLIS BIODYNE, TARTU-ESTONIA) and 12 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. GSTM1 genotypes were determined by the presence and absence (null) of bands.

RESULTS

this case control study includes 100 participants, 50 of them were Sudanese patients with CLL and 50 apparently healthy volunteers were included in the study as control group. The patients ages were ranged from 21-70 years (mean46). the age of control group subjects were ranged from 30 to 60 years(mea40).

The GSTM1 null polymorphism was detected in 38% of cases and 36% of control subjects (table1) but the difference was statistically significant (or=2.9 95% C l = 1.28 -6.5, p=0.01) therefore GSTM1 null genotype may be a risk factor for CLL. (P. value =0.01).

DISSCUSSION AND CONCLUSION

Homozygote's for the null alleles (deletion) of GSTM1 lack activity of the respective enzymes (strange and fryer 1999) 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 (kim et al 2000). In our study we conclude that the GSTM1 null genotype was found to be significance association for increasing CLL risk (OR= 2.9 95% CL=1.28-6.5 P=0.01) and without relation in ages. this finding is in agreement with other studies, in which other cancers were studied. A study in India on leukemic patients showed significance association of GSTM1 with acute lymphoblastic leukemia (ALL) (Akane naruoka et al 2014). in contrast also many studies showed negative result in the association between GSTM1 null genotype and various type of diseases and cancer. A study in china on leukemic patient showed no significance association of GST with acute myeloid leukemia (AML) acute non lymphoblastic leukemia (ANLL) and chronic myelogenous leukemia (CML) (chen et al 2008).

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