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RESEARCH ARTICLE

GSTT1 GENE A PROTECTIVE FACTOR FORM CHRONIC MYELOID LEUKEMIA AMONG SUDANESE POPULATION

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Abbreviation:

CML: Chronic Myeloid Leukemia,
GST: Glutathione –s- transferase,
PCR: polymerase Chain Reaction,
GSTT1: Glutathione –s- transferase T1,
TWBCs: Total white blood cells**.**

ABSTRACT

Back ground: Interaction of environmental and genetic elements plays important role in the pathogenesis of CML and other types of cancer. Glutathione S-transferase (GST) is adetoxifying enzyme. Absence or low levels of this enzyme may genetically predispose individuals to CML. The aim of the present study was to determine GSTT1 Polymorphism among Sudanese patients with Chronic Myeloid Leukemia (CML) attending Radiation and Isotope Center-Khartoum.
Materials and methods: Cross sectional case control study was conducted between January 2016 and August 2016 , on 50 patients with chronic myeloid leukemia among both genders at different ages admitted to Radiation and Isotope Center-Khartoum and 50 apparently healthy control subjects. The GSTT1 genotype was determined using polymerase chain reaction (PCR) method
Result: The percentage of GSTT1 null genotype in CML patients was significantly higher than control (OR = 4.00, 95% CI: 1.62 –9.82; p =0.001).while GSTT1 polymorphism shows no statistically significance with the CML phases and hematological parameters except with the TWBCs.
Conclusion: GSTT1 may be a protective factor for CML, while the null genotype shows association with the developing of chronic myeloid leukemia.

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INTRODUCTION

Leukemia is a group of cancers that usually begin in the bone marrow and result in high numbers of abnormal white blood cells (Leukemia, 2014). These white blood cells are not fully developed and are called blasts or leukemia cells (Hutter, 2010). Symptoms may include bleeding and bruising problems, feeling tired, fever, and an increased risk of infections (Hutter, 2010). These symptoms occur due to a lack of normal blood cells. Diagnosis is typically made by blood tests or bone marrow biopsy (Hutter, 2010). The exact cause of leukemia is unknown. Different kinds of leukemia are believed to have different causes. Both inherited and environmental (non-inherited) factors are believed to be involved (A Snapshot of Leukemia, 2014). Risk factors include smoking, ionizing radiation, some chemicals (such as benzene), prior chemotherapy, and Down syndrome (A Snapshot of Leukemia, 2014 and World Cancer Report, 2014). People with a family history of leukemia are also at higher risk (World Cancer Report, 2014). There are four main types of leukemia :acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) as well as a number of less common types (World Cancer Report, 2014 and SEER Stat Fact Sheets: Leukemia, 2011). The glutathione S-transferases (GSTs) are a family of enzymes belonging to phase II enzymes involved in detoxification of xenobiotics. A significant relationship is observed between the risk of developing cancer and genetic polymorphisms within GSTs (American Cancer Society, 2014). Xenobiotic metabolizing enzymes (XMEs) constitute one of the first lines of defense against environmental chemicals. They play a central role in the metabolism, elimination, and detoxification of xenobiotics or exogenous compounds introduced into the body (Martin Stanulla, 2000). Cells have

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developed an effective mechanism to prevent accumulation of damaging xenobiotics by way of their elimination catalyzed by multiple enzyme system. The enzymes of the multiple enzyme system are classified in two categories namely Phase I and Phase II. Phase I enzymes like Cytochrome P450 can activate the carcinogens directly and produce active metabolites while Phase II enzymes like glutathione-S-transferase (GSTs) can detoxify and process the activated metabolites for final breakdown (Bhat, 2012). Inherited absence of alleles (null genotype) in GSTT1genes result in lack of enzymatic activity (9) the frequencies of GSTs polymorphic alleles, especially GSTT1 and GSTM1, have been reported in various cancers. The aim of this study was to to determine GSTT1 Polymorphisms among Sudanese patients with Chronic Myeloid Leukemia.

MATERIALS AND METHODS

Subjects

This prospective cross-sectional case control study was carried out at RICK, the study was conducted between January and september 2016. During the study period a Total of 100 individual (50 known chronic myeloid leukemia patients and 50 apparently healthy individuals as control) were enrolled in the study. After This study was performed after approval of the local Ethics Committee and individual informed consent from patients and control. DNA was extracted by Chelix (100) method protocol (Sean Walsh, 2013); Samples were stored at -20C until analysis, Gene quant device (Amersham bioscience – Biochrome LTD- Cambridge CB4 of J. England) used to detect the quantity of (DNA& Protein) and quality (Purity &ratio) of DNA.

Genotyping of GSTT1 polymorphism

Allele specific polymerase chain reaction (Bio-RAD – Mexico) 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-TTC CTTACTGGTCCTCACATCTC-3, Antisense primer: 5-TCACCGGATCATGGCCAGCA-3. PCR was carried out in a total volume of 25 µl. It consist of 5µl of genomic DNA, 1µl from each primer, ready to load master mix (Maxime PCR premix series) and 11µl distilled water. PCR was initiated , initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 61°C for 1 minute, 72°C for 1 minute and a last extension at 72°C for 7 minutes. PCR products were analyzed on a 2% Agarose gel stained with 0.5 µg/mL ethidium bromide, and visualized by gel documentation system. 100 BP DNA ladder *(Vivantis 100 pb plus, 0.1 µg /µl) was* run with each batch of patients' and control 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 489 BP, since the assay does not distinguish heterozygous or homozygous wild-type GSTP1 was also tested (as internal control) because the absence of GSTT1 amplification product in the presence of the GSTP1 PCR product indicates a GSTT1-null genotype. The GSTP1 primers used were: 5'-GTA GTT TGC CCA AGG TCA AG-3' (F) and 5'-AGC CAC CTG AGG GGT AAG-3' (R).

RESULTS

A total of 50 patients diagnosed with chronic myeloid leukemia attended to the Radiation and Isotope Center of Khartoum (RICK) Sudan, their ages ranged between (8- 78) years (mean±SD:38.1±18.6), 25(50%) of them were males and 25(50%) were females, compared to 50 volunteers as control group their ages ranged between 18-50 years, 27(54%) of them were males and 23(46%) of them were females. The frequency of GST (T1) comprised 30 (60%) among patients and GST (T1null) comprised 20(40%) of patients, while GST (T1) and GST (T1null) comprised 45(90%), 5(10%) of control respectively.

Table 1. Descriptive statistics for GSTT1 among patients and control group

Parameter	Patients	Controls	P.value
Age, mean (range)	38.1 (8- 78)	26.9 (18-50)	
Gender			
Male	50% (25/50)	54% (27/50)	-
Female	50% (25/50)	46% (23/50)	
Gene variants			
GSTT1(Null)	40% (20/50)	10% (5 /50)	0.001
GSTT1(present)	60% (30/50)	90% (45/50)	

paerson`s chi- squire test was used to calculate P value
P value less than 0.05 considered significant

Table 2. Descriptive statistics of GSTT1 Among different CML phases in patients group

Phase	Gene Varient		OR with 95% CI	Totlw	P value
	Null	Positive			
Chronic	17	23	1.10	40	0.365
Accelirated	3	6	0.64	9	
Blast	0	1		1	
Total	20	30	1.72	50	

paerson`s chi- squire test used to calculate P value
P value less than 0.05 considered significant

Table 3. Descriptive statistics of GSTT1 polymorphism and hematological parameters among CML patients

Parameter	Gene variant				p. value
	T1 Null	Mean	T1 positive	Mean	
Hb (g/dl)	20	11.2	30	11.7	.080
Platelets ($X10^9$/L)	20	10395.00	30	25733.33	.721
TWBCs ($X10^9$/L)	20	265.15000	30	243.56667	.023
Basophil (%)	20	0.6	30	.27	.126
Eosinophil (%)	20	.95	30	1.93	.328

Independent t test was used to calculate P value
P value less than 0.05 considered significant

The statistical analysis showed that there was a significant difference in the frequency of Glutathione S-transferase-T1 gene between patients and control group. In addition, the percentage of GSTT1 null genotype in CML patients was significantly higher than in control (OR = 4.00, 95% CI: 1.62 – 9.82; p =0.001). Figure (4.1). Concerning GSTT1gene polymorphism pattern in different CML phases among all study patients there was a statistically insignificant difference regarding the GSTT1 null genotype between CML phases. The present study showed that chronic phase was more common in patients harboring this mutant type than in both accelerated phase and blast crisis (OR = 1.72, 95% CI: 0.38-7.65; p 0.365). Also the result of GSTT1 polymorphism among Sudanese tribes showed that the mutant gene is higher in western tribes (11 % of all study group) and lower frequency in eastern tribes (0%).

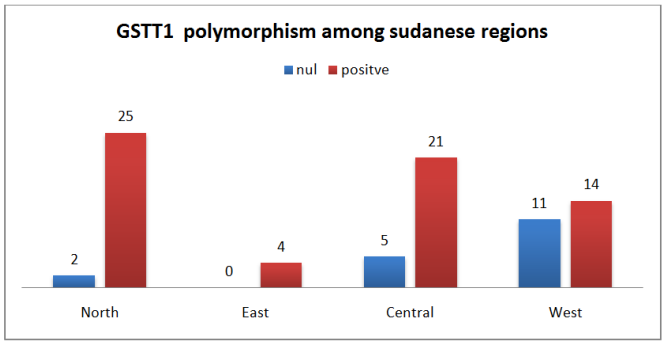

Figure 4.1. GSTT1 polymorphism among Sudanese regions

DISCUSSION

CML is a myeloproliferative disorder but definite mechanism leading to this carcinogenesis is not completely understood yet. [(11)] Genetic susceptibility studies of CML may serve to identify populations at risk and clarify important disease mechanisms. Genetic variants within genes that encode enzymes involved with metabolism such as GST have been shown to increase the likelihood of developing various forms of cancers (Kiran, 2010). This study was performed to investigate the association between the Glutathione S-transferase gene (T1) and (CML) in Sudanese patients .the study found that association between GST (T1null) and CML risk) OR = 4.00, 95% CI: 1.62 –9.82; p =0.001). This finding was agreed with study done by Ovsepyan *et al*. which showed that the individuals with GSTT1 null genotypes may have an increased susceptibility to develop CML(OR=3.66, 95% CI=2.12-6.30; p<0.0001). The present study also was agreed with study done by Sara kamil, *et al*, which showed that, GSTT1 null genotype was significantly associated with the risk of CML in males (OR 95% CI, 5, 1.25-20.1; p = 0.023) (Sean Walsh, 2013). Our study was disagreed with Claudia Bănescu, *et al* who suggested that No association was observed between CML and GSTT1 polymorphisms in any of the investigated cases (Claudia Bănescu, 2014). Our study noticed that null gene of GSTT is higher in western population while it is absent among eastern, no previous data was found on this result. Our study also revealed there was no statistically relation between the absence or presence of the gene and the disease phases or clinical outcome, this was disagreed with Shereen Elhoseiny, *et al*., (Shereen Elhoseiny, 2014), and this may relate to the imbalance distribution of the phases (chronic 80 %, accelerated 18% and blast 2%) and or low sample size.

Conclusion

The percentage of GSTT1 null genotype in CML patients was significantly higher than in control, Therefore, GSTT1 genotype may be a protective factor for CML , while the null genotype show association with developing of chronic myeloid leukemia

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