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DELETION OF GSTT1 GENE POLYMORPHISM AS A RISK FACTOR FOR DEVELOPMENT OF CHRONIC MYELOID LEUKEMIA

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ABSTRACT

Background: Chronic myeloid leukemia (CML), as most of cancers results from complex interaction between genetic or non genetic factors. Exposures to xenobiotics endogenous or exogenous associated with a reduced individual ability in detoxifying activity, constitutes a risk of developing cancer. It is known that polymorphism of glutathione S-transferases (GSTs) genes affects the detoxification of xenobiotics. **Materials and Methods:** Thus, we conducted a case-control study in which 50 patients (Mean age ± SD, 45.0 ± 12.9 years) with CML and 93 healthy unrelated controls (Mean age ± SD, 38.2 ± 10.3 years) have participated. GSTT1 genotypes were determined by polymerase chain reaction. Logistic regression was used to assess the possible link between GSTT1 null genotypes and CML as well as between combined genotypes and CML. **Results:** GSTT1 null genotype frequency was slightly higher in patients than control (50.0% vs. 16.7%) but, it was not associated with CML (OR 95% CI, 1.4, 0.78-2.48; p = 0.271). Surprisingly, GSTT1 null genotype was significantly associated with the risk of CML in males (OR 95% CI, 5, 1.25-20.1; p = 0.023). In summary our findings have shown that GSTT1 null genotype might be a risk factor of CML. However, further studies with a large sample size are needed to confirm our findings.

KEY WORDS:

INTRODUCTION

Glutathione S-transferases (GSTs) are broadly expressed in mammalian tissues. These enzymes have wide substrate specificity and polymorphic genes e.g. GSTT1, GSTP1 and GSTM1 (Joseph. *et al.*, 2004). The role of GSTT1 and GSTM1 in cancer has already been well documented. Polymorphisms in the GST enzyme family have been associated with survival and toxicity issues in children and adults who have leukaemia (Yuille et al., 2002). These genes coding for enzymes that play a main role in the metabolism of xenobiotics, including chemotherapeutics, reactive species and environmental carcinogens it would be useful to know about these polymorphisms in Sudanese patients with CML.

GSTs are from a super-family of genes whose products are phase II enzymes. GSTM1 and GSTP1 detoxify carcinogenic polycyclic aromatic hydrocarbons, while GSTT1 is responsible for the detoxification of smaller reactive hydrocarbons, e.g. ethylene oxide (Malone et al., 1989). The GSTP subfamily comprises only GSTP1. Differences in activities of some GSTs are determined by genetic polymorphisms. GSTM1 activity is absent in 50% of whites as a consequence of the inheritance of 2 null alleles (gene deletion). GSTT1 activity is also deficient in a further 20% of whites, resulting from homozygous deletion. The 1578A_G substitution in GSTP1 generates the Ile105Val polymorphism that results in an enzyme with reduced activity. There is epidemiologic evidence that exposure to aliphatic or a chlorinated hydrocarbon has a role in CGL aetiology. This, coupled with the proposed role of GSTs in the cause of a number of common cancers gives a strong rationale for evaluating GSTM1, GSTT1, and GSTP1 polymorphisms as risk factors for CGL (Lourenco et al., 2005).

In Sudan, there is no literature published on GSTT1 deletions in CML. Thus the aim of this study was to evaluate GSTT1 polymorphism as risk factors for CML.

MATERIALS AND METHODS

This was a prospective case-control study included 50 Sudanese patients with CML attended to Radiation and Isotope Centre of Khartoum in the period from May to December 2015 and 30 apparently healthy volunteers as control group . Blood samples (3ml) were collected from patients and control- in ethylene diamine tetra acetic acid (EDTA) containers.

DNA extraction: Genomic DNA was extracted from blood sample by using salting-out method (Miller et al. 1988) and the yield was checked for quality by running in 1% agarose gel electrophoresis, then DNA samples were stored below -20c until analysis.

Genotyping of GSTT1 null polymorphism
Allele specific polymerase chain reaction was used for detection of the polymorphic deletion of GSTT1 null polymorphism.

Primer direction	Sequence	Product size (bp)
Forward primer	ATC TC3'	480
Reverse primer	5'TCA CCG GAT CAG GCC AGCA3'	

PCR was performed in total volume of 20µl containing 4µl of genomic DNA,1µl of each primer Tle (1) ,14µl of Distilled water all these ingredients were put in pre mixed tube(Maxime PCR premix kit(I-Taq).

Thermocycling conditions includes denaturation step at 94^0C for 10 minute followed by 35 cycles of denaturation at 95^0C for 1minute ,annealing at 62^0C for 1minute and extension at72^0C for 1 minute and followed by final extension at 72^0C for 10 minute.

After amplification in (TC-412, UK) thermocycler ; PCR products were analyzed using 2% agarose gel containing ethidium bromide and also 100bp DNA ladder was run with each batch of patients samples and visualized under UV transilluminator by gel documentation system (SYNGENE, JAPAN). GSTT1genotypes were determined by the presence and absence(null) of band of 480pb.

Statistical analysis: Logistic regression was used to assess the risk between *GSTT1* null genotypes and the occurrence of CML. Odds ratio (OR) with a confidence interval (CI) of 95% was calculated. The chi-square test was used to compare the genotype distribution between patients and control. *A p-value* less than 0.05 was considered as statistically significant. We have used the statistical package SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Ethical considerations: All patients and controls sampled in the study signed an informed consent approved by the Human Ethics Committee of Al Sudan Unversity.

RESULTS

This case control study includes 80 participants, 50 of them were Sudanese patients with CML and 30 apparently healthy volunteers were included in the study as control group. The patients' ages were ranged from 19 to 67 years (Mean 45). The ages of control group subjects were ranged from 25 to 55 years (Mean 38.2years). 32 (64%) of patients were males and 18(36 %) of them were females where as 22 (73.3%) of controls were males and 8 (26.7%) were females. The frequency of individuals carrying the GSTT1 null mutation was higher in CML patients (50 %) compared to controls (17%) (OR=5.0, 95% CI= 1.4- 8.4, p= 0.003).Therefore GSTT1 null genotype may be a risk factor for CML. The gender distribution of the overall GSTT1 null genotype among the study group was 38.9% males and 34.6 % females and there is no significant association between gender and the presence of the polymorphism (P.Value: 0.7).

GSTT1 Genotype	Patient	Control	OR	P.V
GSTT1 Null	25 (50%)	5(17%)	3.5	0.003
GSTT1 Present	25 (50 %)	25 (83%)		

DISCUSSION

Homozygotes for the null alleles (deletion) of GSTM1 and GSTT1 lack activity of the respective enzymes (Strange, R. C) 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, J.W.,).
The current study, the GSTT1 null genotype frequency (50 %) was comparable to that observed in Asians (35-52%) but higher than that observed in Caucasians (10-26%) (Garte et al. 2001; Van der Logt et al. 2004). In the current study, we noticed that the GSTT1 null genotype was higher in patients (50 %) than in control (17%) it was associated with an increased risk to develop CML (OR 95% CI, 3.5, 1.4-8.4; p = 0.003) (Table 2).

CONCLUSION

In summary we have noted that the GSTT1 null genotype is associated with the development of CML.

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