Case Report

Glutathione S-transferase M1 (GSTM1) Polymorphism and Childhood Acute Lymphoblastic LeukemiaRisk: A Case-Control Study- Khartoum-Sudan(2014-2017)

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Abstract

Background: Glutathione S-transferase (GST) enzyme levels are associated with the risk of many cancers, including hematological malignancies. Conflicts in the published results and the absence of similar in-depth studies in Sudan prompted us to perform the present investigation to detect a possible association between polymorphisms at the GSTT gene and susceptibility to develop ALL in a Sudanese population.

Objectives: This study aimed to investigate the relationships between GSTT1 polymorphisms and the risk of acute lymphoblastic leukemia (ALL).

Materials and Methods: A total of 40 patients with ALL and 30 control subjects were enrolled in this case control study. Blood samples were collected from all patients and controls in EDTA. Genomic DNA was extracted from all blood samples using salting out method. Genotyping for detection of GSTT1 polymorphism was performed for both patients and controls using an allele-specific PCR

Results: GSTT1 null genotype was detected in 53% of ALL patients (21/40) and in 17% (5/30) of the control group, and GSTT1 present genotype was detected in 47% (19/40) in ALL patients group and in 83% (25/30) of the control group. We reported that there is an association between the GSTT1 null genotype and ALL risk (OR= 5.5, 95% CI= 1.7616 to 17.3364; P = 0.003).

Conclusion: This study reported that there was an association between the GSTT1 null genotype and ALL risk(OR=5.5, 95% CI=1.7616 to 17.3364; P=0.003) appear also to have an influence in the development of ALL.

Keywords: ALL- GSTT1- genetic polymorphism, Risk

Introduction

Glutathione S-transferases (GSTs) are a supergene family of detoxifying enzymes which are found in virtually all life forms [(1)]. GSTs are phase II detoxifying enzymes that catalyze the conjugation of reduced glutathione with a wide variety of electrophilic substrates [(2)]. In addition to their function in xenobiotic detoxification, GSTs have peroxidase and isomerase activities that can inhibit the c-Jun N-terminal kinase (JNK) [(3)]. GSTs can also bind non-catalytically with a wide range of endogenous and exogenous ligands [(3)]. In humans, GST enzymes consist of many cytosolic, mitochondrial, and microsomal proteins, and the cytosolic family has eight distinct classes: alpha (A), kappa (K), mu (M), omega (O), pi (P), sigma (S), theta (T), and zeta (Z) [(4, 1)]. Among the numerous GST genes, GSTM1 and GSTT1 genes have been extensively studied because of the high prevalence of homozygous deletions of these genes, which have been associated with the loss of enzyme activity with a decreased ability to detoxify and increase vulnerability to cytogenetic damage, placing null individuals at increase cancer risk [(5-7)]. GSTT1 is represented by two alleles: a functional or wild allele (GSTT1*1), and a nonfunctional or null allele (GSTT1*0). Studies have shown that the total or partial deletion of the gene (GSTT1*0 allele) causing a deficiency in its enzymatic activity [(8)]. Moreover, numerous studies have evaluated the association between polymorphisms of GSTs gene and the susceptibility to develop various types of cancer, such as gastric cancer, oral cancer, bladder cancer, and chronic myeloid leukemia CML in different ethnical groups worldwide [(9)].To the best of our knowledge this is the first study conducted in Sudan to investigate the association between the GSTT1 polymorphism in ALL patients. The present study was conducted to detect a possible association between polymorphisms at the GSTT gene and Risk of ALL. in a Sudanese population.

Materials and Methods

In this case-control study a total of forty patients newly diagnosed with ALLwere recruited from the Radio Isotope Center Khartoum (RICK) Sudan in the period from October 2014 to March 2015. Additionally, thirty healthy age and gender-

matched children served as control group.

Ethics approval and consent to participate

The authorized guardians of both patients with ALL and controls had given informed written consent after clarifying the nature, steps, and aim of the study. The study was conducted with the approval of the Ethics Committee of Sudan University of Science and technology.

Blood sample collection.

Peripheral blood samples were obtained by venipuncture using a sterile aseptic technique. Four ml of peripheral Blood samples were collected in EDTA tube for DNA extraction and genotyping.

DNA extraction

Genomic DNA was extracted by the salting out method(10). To assess the DNA quality after DNA extraction, the β-globin gene amplification was used to evaluate the quality of DNA in all extracted samples, as previously described(11). All specimens for β-globin gene showed successful amplification, (Primers shown in Table 1). To evaluate the DNA quantification after DNA extraction, we measured DNA by using a NanoDrop spectrophotometer.A260:280 ratios greater than 1.8 were considered indicators for highly pure DNA. Then DNA samples were routinely stored at -20°C.

Genotyping of GSTT1 polymorphism

Genotyping was carried out by using the polymerase chain reaction with allele specific primers as described previously (12). The primer sequences for genotyping are shown in Table 1. PCR was performed by using *Maxime PCR Premix Kit (i-Taq), (iNtRON BIOTECHNOLOGY*, *South Korea*), Cat. No. 25025), 4µl of genomic DNA, 0.5 µl of each primer, and 14 µl distilled water. The thermal profile was 94°C for 5 minutes as initial denaturation, followed by 35 cycles each cycle of 95°C for 60 seconds, 60°C for 60secnds, and 72°C for 60 seconds, with a final extension at 72°C for 5 min. Thermo cycling was carried out on the TECHNE Tc-412-UK PCR Thermal Cycler 96 well (TECHNE, UK).Amplified

products were electrophoretically separated through 1.5 % agarose gels and 0.5X TBE buffer and visualized under UV light with ethidium bromide staining using Dolphin Doc gel documentation system

GSTT1 genotype was specified by bands of 480 bp, the GSTT1 Null genotype showed one band of 480 bp, hence the use of β-globin gene as internal positive control was important to identify the null genotype from aborted PCR responses.

Table (1):The primers sequence forGSTT1 polymorphismsG Polymorphism

Primer	Sequence	Product size (bp)
GSTT1 forward	5'-TTCCTTACTGGTCCTCACATCTC-3',	480 bp
GSTT1 reverse	5'-TCACCGGATCATGGCCAGCA-3'	
ß-globin forward	5'-CAACTTCATCCACGTTCACC-3',	268 bp
ß-globin reverse	5'-GAAGAGCCAAGGACAGGTAC-3	

Statistical analysis

Patient's data was collected by structured interview questionnaire and from patients medical files and analyzed using the statistical package for social sciences (SPSS) version 16.0 software (Chicago, IL, USA). Logistic regression was used for calculation of odds ratio (OR) with confidence interval (CI) for risk estimation

Results

A total of 70 study subjects comprising forty ALL patients (19 males, 11 females, mean age 12.2 years) and thirty healthy (29 males, 11 females, mean age 14.2 years) controls were studied. The frequencies of GSTT1 null genotype were higher in ALL patients 53% (21/40) than in control`s group 17% (5/30). However, for the GSTT1 precent genotype frequencies were 47 % (19/41) in patients' group and 83% (25/30) in controls' group. Statistically, significant difference was observed (OR= 5.5, 95% CI= 1.7616 to 17.3364; P.value= 0.003) (Table 2). In ALL patients GSTT1 was null in 38% of males and 13% of females and presented in 33% of males and 16% of females whereas in control group GSTT1 was null in 4% of males and 13% of females and presented in 63% of males and 20% of females.

Table 2: Frequency of GSTT1 genotypes in ALL patients and controls

Group	ALL Patients	Control	Odd Ratio	*P. Value*	95% CI
GSTT1 Null	47%	17%	5.5	0.003	1.8-17.3
GSTT1 Present	53%	83%			

Discussion

Glutathione s-transferase (GSTs) polymorphisms (GSTM1, GSTP1 and GSTT1) have been reported to be risk factors for developing solid cancers and leukemias in many published reports; several studies reported the association between GSTT1 polymorphisms and risk of childhood acute lymphoblastic leukemia.Genetic variability of GST genes was evaluated in leukemic and healthy Sudanese since ethnicity has been related to differences in the occurrence of acute leukemia (13), and notable variances in the pattern of GST frequencies were described worldwide (14,15).In this case-control study, we found that the GSTT1 deletion polymorphisms were associated with risk of ALL, this finding is like that found in earlier studies on childhood ALL patients. However, the odds ratio obtained in this study is much higher than in the previous studies (16). The findings of the percent study are partially agreeing with study done in India (17) which demonstrated that GSTT1 null genotypes may be considered independent risk factors for ALL. This study indicated GSTT polymorphism was a promising candidate biomarker for evaluating the ALL risk. There are several previous studies which investigated the association between GSTT1 polymorphisms and risk of ALL (16, 18), but the results are different. There is a case-control study conducted in the Singapore by Chan et al, and the results support the hypothesis that the GSTT1 gene deletion is related to the risk of ALL (19). Clavelhas reported that the variation in GSTT1 genotype was not associated with the susceptibility of risk of development of ALL in a French population (20). The results obtained in our study indicating that GSTT1 null genotype is a risk factor for childhood leukemia agree with earlier publications in Sudan (21) indicating that GSTT1 null

genotype may increase individual susceptibility to AML.

The explanations for the variations between different studies are not clear. However, some clarifications can be proposed including that the differences may be due to variable geographical distribution and some interethnic variances may be involved

Conclusion

These results imply that genetic variants of GSTT1 gene influence the risk of developing childhood ALL.

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References

1. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol. 2005; 45, 51-88.
2. Hayes JD, Pulford, DJ. The glutathione S-transferase supergene family: Regulation of GST and the contributions of the isoenzymes to cancer chemoprotection and drug resistance. CRC Crit Rev Biochem Mol Biol. 1995;30, 445-600.
3. Benabdelkrim M , Djeffal O , Berredjem H. GSTM1 and GSTT1 Polymorphisms and Susceptibility to Prostate Cancer: A Case-Control Study of the Algerian Population. Asian Pacific Journal of Cancer Prevention. 2018; 19(10), pp.2853-2858.
4. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. Mutat Res. 2001; 482, 21-6.
5. Bolufer P, Barragan E, Collado M, et al. Influence of genetic polymorphisms on the risk of developing leukemia and on disease progression. Leuk. Res. 2006; 30: 1471-91.
6. Bolt HM, Thier R. Relevance of the deletion polymorphisms of the glutathione S transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr Drug Metab. 2006; 7: 613-28.
7. Al-Sarraji FAA. Molecular and cytogenetic studies of human populations in two districts of Baghdad. PhD thesis, College of Science, Baghdad University, Baghdad, Iraq, 2013.
8. Pemble S, Schroeder KR, Spencer SR, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J. 1994; 300: 271-6.
9. Ma W, Zhuang L, Han B, et al. Association between glutathione S-transferase T1 null genotype and gastric cancer risk: a meta-analysis of 48 studies. PLoS One. 2013; 8(4): e60833.
10. Miller S, Dykes D, Polesky H: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids research 1988, 16(3):1215.
11. Kerr J, Al-Khattaf A, Barson A, Burnie J: An association between sudden infant death syndrome (SIDS) andHelicobacter pylori infection. *Archives of disease in childhood* 2000, **83**(5):429-434.
12. Abdel-Rahman S, El-Zein R, Anwar W, Au W. A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. Cancer Letters. 1996;107(2):229-233.
13. MetayerC, Milne E, ClavelJ, Infante-RivardC, PetridouE, Taylor M, *et al.*The Childhood Leukemia International Consortium. Cancer Epidemiol. 2013;37:336–47.
14. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, *et al.* Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev. 2001;10:1239–45.

15. Piacentini S, Polimanti R, Porreca F, Martinez-Labarga C, De Stefano, Fuciarelli M. GSTT1 and GSM1 gene polymorphisms in European and African populations. MolBiol Rep
16. Krajinovic M, Labuda D, Richer C, et al. Susceptibility to childhood acute lymphoblastic leukemia: Influence of CYP1A1, CYP2D6, GSTM1 and GSTT1 genetic polymorphisms. Blood 1999; 93:1496–1501.
17. Joseph T, Kusumakumary P, Chacko P, Abraham A, Radhakrishna Pillai M. Genetic polymorphism ofCYP1A1,CYP2D6,GSTM1 andGSTT1 and susceptibility to acute lymphoblastic leukaemia in Indian children. Pediatric Blood & Cancer. 2004;43(5):560-567.
18. Sinnett D, Krajinovic M, Labuda D.Genetic susceptibility to childhood acute lymphoblastic leukemia. Leuk Lymphoma2000; 38: 447–462.
19. Chan JY, Ugrasena DG, Lum DW, Lu Y, Yeoh AE (2011) Xenobiotic and folate pathway gene polymorphisms and risk of childhood acute lymphoblastic leukaemia in Javanese children. Hematol Oncol 29: 116-123
20. Clavel J, Bellec S, Rebouissou S, Ménégaux F, Feunteun J et al. (2005) Childhood leukaemia, polymorphisms of metabolism enzyme genes, and interactions with maternal tobacco, coffee and alcohol consumption during pregnancy. Eur J Cancer Prev 14: 531-540.
21. Ahmed Babekir E, Mahmoud Abdelateif N, Osman Adelrahim S, Khider Ibrahim I. GSTM1 and GSTT1 Polymorphisms and Susceptibility to Acute Myeloid Leukemia: A Case-control Study of the Sudanese Population. Asian Pac J Cancer Biol. 2019;4(1):7-10.