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FREQUENCY OF GSTM1 AND GSTT1 GENE POLYMORPHISMS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AND THEIR ASSOCIATION WITH HEMATOLOGICAL PARAMETERS

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ABSTRACT

Background: Genetic polymorphisms of Glutathione S-transferase Mu 1 (GSTM1) and Glutathione S-transferase Theta 1 (GSTT1) have been associated with altered detoxification capacity and may contribute to the development of childhood acute lymphoblastic leukemia (ALL).

Objective: To determine the frequency of GSTM1 and GSTT1 polymorphisms among children with ALL and evaluate their association with hematological parameters.

Methods: A Case-control study was conducted among 45 children with ALL cases and 45 healthy controls. GSTM1 and GSTT1 genotypes were detected using multiplex polymerase chain reaction (PCR) with β-globin as an internal control. PCR amplification was performed using 2× EasyTaq® PCR SuperMix (TransGen Biotech, UK). The observations on hematological parameters such as hemoglobin, total leukocyte count, platelet count and differential leukocyte count were obtained and analyzed.

Results: A total of 90 participants (45 ALL patients and 45 healthy controls) were included in the study. Hematological abnormalities typical of leukemia were seen in ALL patients with increased WBC, blast and decreased Hb and platelet levels ($p < 0.05$) when compared to controls. With regards to genetic analysis, the GSTM1 null genotype was significantly associated with childhood ALL susceptibility ($p = 0.027$), while the GSTT1 null genotype showed a borderline, non-significant association with childhood ALL susceptibility ($p = 0.056$). In subgroup analysis, GSTT1 showed significant associations, which implies that it may be involved in disease presentation instead of overall disease susceptibility.

Conclusion: There was a significant association between GSTM1 null genotype and childhood ALL, but not for GSTT1 which had only a weak, non-significant association. No consistent association was found between the GST polymorphisms and the hematological parameters, but in the case of GSTT1, significant association was observed with blast percentage and easy bleeding in the subgroup analysis.

KEYWORDS: Childhood Acute lymphoblastic leukemia; GSTM1; GSTT1; genetic polymorphism; hematological parameters; glutathione S-transferase; leukemia susceptibility; PCR.

INTRODUCTION

Childhood Acute lymphoblastic leukemia (ALL) is a malignant disorder of the hematopoietic system characterized by clonal proliferation and accumulation of immature lymphoid progenitor cells in the bone marrow, peripheral blood, and extramedullary sites. It is the most common childhood malignancy, but it also occurs in adults with more aggressive clinical behavior and poorer prognosis (1). Despite advances in chemotherapy and supportive care, the etiology of childhood ALL remains incompletely understood, and it is widely accepted that both genetic susceptibility and environmental exposure play a critical role in disease development (2).Leukemogenesis is a multistep process involving genetic alterations that disrupt normal hematopoiesis, including activation of oncogenes, inactivation of tumor suppressor genes, and impaired DNA repair mechanisms (3,4). Among the various genetic factors implicated in cancer susceptibility, polymorphisms in detoxification enzymes have gained significant attention. Glutathione S-transferases (GSTs) are a family of phase II metabolic enzymes responsible for detoxifying electrophilic compounds, including carcinogens, environmental toxins, and oxidative stress by-products through conjugation with glutathione (5,6).

The GST family includes several classes, among which Glutathione S-transferase Mu 1 (GSTM1) and Glutathione S-transferase Theta 1 (GSTT1) are the most widely studied in relation to cancer susceptibility (7). A common genetic variation in these genes is the homozygous deletion (null genotype), which leads to complete absence of enzyme activity. Individuals with GSTM1 and GSTT1 null genotypes are unable to efficiently detoxify carcinogens, resulting in increased DNA damage and genomic instability (8). Multiple epidemiological studies have investigated the association of GST polymorphisms with various malignancies, including lung cancer, bladder cancer, breast cancer, and hematological malignancies. However, findings remain inconsistent across populations due to genetic diversity, environmental exposures, and sample size variations (9). In the context of leukemia, impaired detoxification capacity may enhance susceptibility to DNA damage induced by environmental carcinogens such as benzene, pesticides, and radiation, all of which have been implicated in leukemogenesis (10).

Childhood Acute lymphoblastic leukemia is characterized not only by malignant proliferation of lymphoblasts but also by profound alterations in hematological parameters, including anemia, thrombocytopenia, and leukocytosis. These changes reflect bone marrow infiltration and suppression of normal hematopoiesis. However, limited data exist regarding the influence of GSTM1 and GSTT1 polymorphisms on hematological profiles in childhood ALL patients, particularly in South Asian populations. Pakistan represents a genetically diverse population with significant exposure to environmental pollutants, occupational chemicals, and socioeconomic risk factors that may contribute to cancer burden. Despite this, limited molecular epidemiological data are available regarding GST gene polymorphisms in hematological malignancies in this region. Hence, understanding the distribution of GSTM1 and GSTT1 polymorphisms and their association with hematological parameters may provide insight into disease susceptibility, progression, and biological behavior of childhood ALL (11). Hence, the present study aimed to investigate the frequency of GSTM1 and GSTT1 gene polymorphisms in children with ALL and compare with healthy controls. Additionally, the study aimed to evaluate the association of these polymorphisms with hematological parameters to explore their potential role in disease severity and clinical presentation.

MATERIAL AND METHODS

This hospital-based case-control study was conducted at Baqai Medical University and National Institute of Child Health (NICH), Karachi, while molecular analyses were performed at the Molecular Biology Laboratory of Mohammad Ali Jinnah University (MAJU), Karachi." Participants were enrolled after obtaining written informed consent from their parents or legal guardians. A total of 90 participants were enrolled, including 45 newly diagnosed, untreated children with ALL patients and 45 age- and sex-matched healthy controls. Sample size was calculated using OpenEpi for unmatched case-control design, and participants were recruited through purposive consecutive sampling based on predefined inclusion and exclusion criteria. A total of 6 mL of venous blood was collected from each participant. The blood was divided equally into two 3 mL ethylenediaminetetraacetic acid (EDTA) tubes; one tube was used for DNA extraction, while the other was used for complete blood count (CBC) analysis. Complete blood count (CBC) parameters were obtained using an automated hematology analyzer.

Genomic DNA was extracted using the GJC DNA purification kit following the manufacturer's protocol. The extracted DNA was assessed for purity via spectrophotometry and agarose gel electrophoresis. GSTM1 and GSTT1 genotypes were detected using multiplex polymerase chain reaction (PCR) with β-globin as an internal control. PCR amplification was performed using 2× EasyTaq® PCR SuperMix (TransGen Biotech, UK) on a Veriti Applied Biosystems thermal cycler, followed by visualization on 3% agarose gel.Data was analyzed using STATA version 17. Quantitative variables were expressed as mean ± SD or median (IQR), and categorical variables as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test. Independent t-test or Mann–Whitney U test was applied for continuous variables, and Fisher's exact test for categorical variables. Odds ratios with 95% confidence intervals were calculated to assess associations, with $p \leq 0.05$ considered statistically significant.

RESULTS

A total of 90 participants were included in the study, comprising 45 childhood Acute Lymphoblastic Leukemia (ALL) patients and 45 healthy controls. The demographic characteristics of the study participants are presented to ensure comparability between the two groups. As shown in Table 1, no statistically significant differences were observed between cases and controls with respect to age and gender distribution ($p > 0.05$). The mean age was comparable in both groups, and the proportion of males and females was also similar, indicating appropriate matching of study participants.

Table 1: Demographic Characteristics of Study Participants (n = 90)

Variable	Cases (n=45)	Controls (n=45)	p-value
Age (Years)	Mean ± SD	Mean ± SD	>0.05
Gender – Male	n (%)	n (%)	>0.05
Gender – Female	n (%)	n (%)	>0.05

Hematological parameters were compared between childhood ALL patients and healthy controls to evaluate disease-related blood profile alterations. As shown in Table 2, significant differences were observed between cases and controls. Childhood ALL patients demonstrated markedly elevated white blood cell (WBC) counts and blast percentages, while hemoglobin and platelet levels were significantly reduced compared to controls ($p < 0.05$), reflecting typical bone marrow suppression associated with childhood all.

Table 2: Comparison of Hematological Parameters between Cases and Controls (n = 90)

Parameter	Cases (n=45)	Controls (n=45)	p-value
WBC (×10⁹/L)	10.5 [5.5–19.7]	5.6 [4.3–8.5]	0.003*
Hemoglobin (g/dL)	8.7 [5.7–9.6]	12.9 [11.6–13.4]	<0.001*
Platelets (×10⁹/L)	43 [25–133]	260.06 ± 89.63	<0.001*

*Significant at $p \leq 0.05$

The frequency distribution of GSTM1 and GSTT1 gene polymorphisms was analyzed in both cases and controls to determine their association with childhood ALL. As shown in Table 3, The GSTM1 null genotype was found to be more common in children with ALL than in healthy controls ($p = 0.027$), suggesting that the genotype is linked with susceptibility to the disease. No association was observed for the null GSTT1 genotype and childhood ALL ($p = 0.056$).

Table 3: Frequency of GSTM1 and GSTT1 Genotypes in Cases and Controls (n = 90)

Genotype	Cases (n=45)	Controls (n=45)	p-value
GSTM1 Present	35 (77.78%)	43 (95.56%)	0.027*
GSTM1 Null	10 (22.22%)	2 (4.44%)	
GSTT1 Present	40 (88.89%)	45 (100%)	0.056
GSTT1 Null	5 (11.11%)	0 (0%)	

*Significant at $p \leq 0.05$

Subgroup analysis was performed among ALL patients to evaluate the association of GSTM1 and GSTT1 polymorphisms with hematological and clinical parameters. The results demonstrated distinct patterns of association, where GSTM1 showed no significant relationship with hematological indices or clinical features, whereas GSTT1 demonstrated significant associations with selected disease-related parameters.

Table 4: Association of GSTT1 Polymorphism with Hematological Parameters and Clinical Manifestations in Children with Acute Lymphoblastic Leukemia (n = 45)

Parameter	P-value
Blast Percentage (%)	0.027*
Easy Bleeding (Yes/No)	0.036*

The study demonstrated significant hematological differences between childhood ALL patients and healthy controls, consistent with disease pathology. GSTM1 null genotype was found to be significantly associated with susceptibility to childhood ALL, while GSTT1 was borderline and not statistically significant.

DISCUSSION

In the present study, the frequency of the GSTM1 and GSTT1 gene polymorphisms was evaluated in children with acute lymphoblastic leukemia (ALL) and its relationship to hematological parameters. In childhood ALL, there was a significantly higher prevalence of the GSTM1 null genotype than in controls, suggesting that the GSTM1 null genotype may be associated with susceptibility to ALL ($p = 0.027$). GSTT1, however, showed a borderline, non-significant association with susceptibility to childhood ALL ($p = 0.056$), indicating a limited involvement in the disease risk in this population (12). Glutathione S-transferase (GST) enzymes play an important role in cellular detoxification processes, detoxifying electrophiles, oxidative stress products and carcinogens. GSTM1 and GSTT1 deletion polymorphisms lead to a complete loss of enzymatic activity and could lead to a decrease in detoxification capacity and DNA damage (13,14). This biological mechanism has been associated with leukemogenesis, especially in populations subject to environmental exposure to toxic chemicals like benzene and pesticides (15,16). The significant association of GSTM1 null genotype in this study suggests that it is a possible determinant of the susceptibility to childhood ALL. As predicted in childhood ALL, differences in the hematological indices were seen between patients and healthy controls, indicating infiltration of the bone marrow and suppressed normal hematopoiesis. Such abnormalities have never been reported as a new observation but are rather typical findings of

childhood ALL and define the study population. The results are in keeping with the known pathogenesis of childhood ALL, which is characterized by bone marrow infiltration with a subsequent impairment of normal hematopoiesis (15). No significant and consistent association was observed between GST polymorphisms and most hematological parameters. However, subgroup analysis demonstrated significant associations between GSTT1 polymorphism, blast percentage, and easy bleeding, suggesting a possible influence on certain clinical or disease-related characteristics rather than overall disease susceptibility. The relationships between the GSTM1 and GSTT1 polymorphisms and leukemia have been found to vary in different populations. Genetic heterogeneity and environmental differences between populations are reflected in some studies that demonstrate significant associations with others showing no relationship (16,17,18). The lack of significant association with GSTT1 found in this study is consistent with other reports indicating a minor contribution to susceptibility to childhood ALL. Differences in the genetic background, in environmental exposures and in the sample size may account for these discrepancies. This study has a few strengths, such as well-matched case-control design with age- and sex-matched participants. The molecular techniques used were standardized and included multiplex PCR that served as a measure of internal control of β-globin (19). Automated hematological analysis increased the confidence of the laboratory results. Furthermore, patients with newly diagnosed and untreated childhood ALL included limiting treatment-related confounding. There are some limitations in the study such as the small number of participants might restrict the generalizability and statistical power of subgroups analysis. No environmental exposure data were collected as this would have an impact on GST enzyme activity. Mechanistic interpretation was limited as functional enzyme activity and markers of oxidation stress were not assessed. Furthermore, research was carried out in a single center, which could limit the generalizability of the results. Larger multicenter group could be a good addition to confirm these results in a wider range of cohorts. Gene environment interaction assessment, especially exposure to environmental carcinogens is recommended. Further studies on the functional activity of GST enzymes and measurement of oxidative stress markers may offer more insight into the mechanisms. Further, the assessment of other genes involved in detoxification could contribute to a more thorough genetic profile of childhood ALL.

CONCLUSION

The present study demonstrated that the GSTM1 null genotype is significantly associated with susceptibility to childhood acute lymphoblastic leukemia, whereas GSTT1 showed only a borderline, non-significant association. Although no consistent relationship was observed between GST polymorphisms and most hematological parameters, GSTT1 polymorphism was significantly associated with blast percentage and easy bleeding in subgroup analysis. These findings suggest that GSTM1 may contribute to childhood ALL susceptibility, while GSTT1 may have a limited role in specific clinical manifestations of the disease.

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