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Frequency of Thiopurine Methyltransferase Gene Polymorphism in Acute Lymphoblastic Leukemia

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Abstract

Objective: To determine the frequency of TPMT gene polymorphism in patients presenting with ALL at NIBD.
Methodology: A cross sectional study was done at the department of Hematology National Institute of Blood Diseases (NIBD) Karachi, from 15th March 2021 to 15th September 2021. A total of 60 patients of either sex presenting within duration of 2 months of diagnosis of ALL were included in the study. Blood samples of all the patients were drawn by senior laboratory technician and sent for Cytogenetic study as well as polymerase chain reaction to identify different alleles of thiopurine methyltransferase gene. The data was entered and analyzed using SPSS version 21. Frequencies and percentages were calculated for categorical variables such as gender, drug toxicity, and allelic variation (TPMT).
Results: Age range in this study was from 14 to 60 years with mean age of 25.733±14.62 years and mean duration of disease was 1.56±0.49 months. Male patients were 80% and females were 20% in this study. Variant TPMT alleles were observed in 10% patients.
Conclusion: The study revealed a 10% frequency of major polymorphisms in 6-MP metabolizing enzymes among Pakistani patients with ALL.
Keywords: Acute lymphoblastic leukemia, TPMT gene polymorphism.

Introduction

Acute lymphoblastic leukemia (ALL) is a rapidly progressing onco-hematological condition characterized by the uncontrolled growth of immature white blood cells in the bone marrow and bloodstream. Patients with different pharmacogenetic variants of ALL experience variable toxic events during therapy, largely due to gene polymorphism.[1,2] The *Thiopurine Methyltransferase* (TPMT) gene, which modifies the metabolism of 6-mercaptopurine (6-MP), has been extensively studied.[3] 6-MP is a purine anti-metabolite used in the treatment of ALL. TPMT deactivates 6-MP into inactive metabolites by catalyzing S-methylation in the cytoplasm.4 The TPMT gene exhibits polymorphism in humans and is inherited as autosomal co-dominant or autosomal recessive.[5] Variations in TPMT are responsible for differences in the therapeutic efficacy and toxicity of thiopurines in individuals.[6] Increased adverse reactions to the same therapeutic dose of thiopurines are observed in patients with polymorphisms that impair TPMT activity.[3,7] Common adverse reactions include recurrent infections due to severe cytopenias, myelosuppression, and treatment failure.[3,8,9] Patients with decreased TPMT activity experience reduced survival rates due to the accumulation of thiopurines to toxic levels.[10] TPMT deficiency and polymorphism can be detected through genetic studies or allele-specific polymerase chain reaction (PCR), both of which have a significant impact on treatment outcomes and dose-related complications of mercaptopurine therapy.[11] Four genetic variants of the TPMT gene have been identified so far: TPMT-2, TPMT 3A, TPMT 3B, and TPMT 3C.[12,13] Statistically, 3.8% of mutant TPMT alleles have been reported.[1] The purpose of this study was to determine the frequency of polymorphisms in the TPMT gene among patients with Acute Lymphoblastic Leukemia (ALL) at the National Institute of Blood Diseases (NIBD). Identifying these polymorphisms will help assess the disease burden and guide the development of appropriate treatment strategies. While international literature provides valuable insights, its direct application may not be feasible due to differences in genetic makeup and geographical variations.

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Methodology

A cross-sectional study was conducted at the Department of Hematology, National Institute of Blood Diseases (NIBD), Karachi. The sample size was calculated using the WHO sample size calculator, based on a proportion of 3.8% for the TPMT allele from a previous study, a margin of error of 3%, and a 95% confidence interval. The required sample size was determined to be 60. Given that around 15 to 20 patients with ALL visit the institute each month, a total sample size of 55 to 60 patients was anticipated over three months. The study was carried out from March 15, 2021, to September 15, 2021, using a non-probability consecutive sampling technique.

Patients aged 1–14 years were classified as children, and those aged 15–60 years were classified as adults. Patients of either sex who presented within two months of an ALL diagnosis (both B-cell and T-cell), confirmed through peripheral blood examination, bone marrow examination, and immunophenotyping, were included in the study. Patients with multiple associated blood-related disorders, those already undergoing treatment, and those with disease recurrence were excluded.

Patients were enrolled from the outpatient department (OPD) and inpatient department once they met the inclusion criteria. All patient data were recorded using a structured proforma, and informed written consent was obtained. Blood samples were drawn by a senior laboratory technician and sent for cytogenetic studies and polymerase chain reaction (PCR) to identify different alleles of the *Thiopurine Methyltransferase* (TPMT) gene. The data was entered and analyzed using SPSS version 21. Frequencies and percentages were calculated for categorical variables such as gender, drug toxicity, and allelic variation (TPMT). Continuous variables such as age and duration of disease were reported as means and standard deviations. Effect modifiers such as age, gender, duration of disease, and drug toxicity were controlled through stratification. Post-stratification, Chi-square/Fisher's exact test was applied, with a p-value ≤ 0.05 considered statistically significant.

Results

The study included 60 participants, with an age range of 14 to 60 years. The mean age was 25.733 ± 14.62 years, and the mean disease duration was 1.566 ± 0.49 months. Male participants accounted for 80% of the sample, while females made up 20%. The frequency and percentage of patients based on drug toxicity are shown in Table III. Variant TPMT alleles were observed in 10% of the patients, as shown in Table I.

Table I: Descriptive statics of demographic and clinical variables. (n=60)

Variables		Statistics	
Age (mean ± SD)		25.733±14.62 years	
Duration of disease		1.566±0.49 months	
Gender	Male	48	80.0%
	Female	12	20.0%
	Total	60	100.0%
Toxicity of Drug	Yes	15	25%
	No	45	75%
	Total	**60**	100%

In this study, the variant TPMT allele was detected in 10% of the 60 participants (Figure. 1).

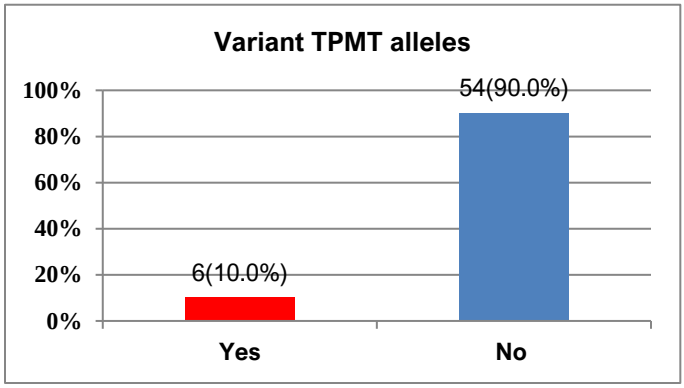

Figure 1. Frequency of variant TPMT alleles. (n=60)

Table II: Stratification of Variant TPMT alleles with respect to age, gender, disease duration and drug toxicity. (n=60)

Variables		Variant TPMT alleles		p-value
		Yes	No	
Age	1-14 years	2(12.5%)	14(87.5%)	0.697
	15-60 years	4(9.1%)	40(90.9%)	
Gender	Male	6(12.5%)	42(87.5%)	0.192
	Female	0(0%)	12(100%)	
Duration of disease	1 month	1(3.8%)	25(96.2%)	0.165
	>1 month	5(14.7%)	29(85.3%)	
Toxicity of drug	Yes	3(20%)	12(80%)	0.136
	No	3(6.7%)	42(93.3%)	

Among children (1-14 years), 12.5% had the variant alleles, compared to 9.1% of adults (15-60 years); however, this difference was not statistically significant (p=0.697). Regarding gender, 12.5% of males had the variant allele, while no females did, but this difference was also not significant (p=0.192). In terms of disease duration, participants with a disease duration of more than 1 month had a higher frequency of the variant (14.7%) compared

to those with a shorter duration (3.8%), although this was not statistically significant (p=0.165). Similarly, the association between the variant allele and drug toxicity was also statistically insignificant (p=0.136) (Table II).

Discussion

Recent clinical guidelines for childhood acute lymphoblastic leukemia (ALL) highlight that the TPMT gene is associated with both the therapeutic effects and side effects of 6-MP. It is identified as a potential candidate for polymorphism testing.[14] This study was initiated due to the growing interest at our institutions in using pharmacogenetics to enhance and personalize leukemia treatment. While our findings affirm earlier reports of a strong correlation between TPMT genotype and phenotype, it is crucial to validate these results within our specific population. This validation is necessary to assess the potential of TPMT genotyping as a diagnostic tool for predicting TPMT activity and the likelihood of 6-MP toxicity at standard doses. The prevalence of TPMT polymorphisms varies among different ethnic groups, ranging from 2% to 14%. In our study of Pakistani patients with acute lymphoblastic leukemia (ALL), we observed an allelic frequency of 10% for the most significant TPMT polymorphisms. This rate is comparable to that reported for blood donors in a previous study.[15] The frequency and distribution of TPMT alleles in Pakistan align with findings from other studies. A key consideration in pharmacogenetics is how specific polymorphisms impact treatment outcomes.

Research has shown that TPMT polymorphisms are linked to variations in 6-MP toxicity and dosag.[16-20] To relate our findings to clinical outcomes, we assessed laboratory parameters and 6-MP dosages in ALL patients who had completed the maintenance phase of their treatment. We found that patients with variant alleles received significantly lower median daily and cumulative doses of 6-MP during the maintenance phase compared to those with wild-type alleles. These results are consistent with previous studies.[21] However, our study did not reveal statistically significant differences in laboratory parameters related to drug toxicities, which may be attributed to adjustments in 6-MP dosage based on ANC values, as recommended by clinical guidelines (20% versus 6%, p=0.136). Overall, these findings underscore the value of TPMT genotyping in identifying high-risk patients who can receive lower doses of 6-MP without affecting the efficacy of ALL treatment.

None of the children with ALL in this study had the TPMT*2 allele. This finding is consistent with previous research from Arabian studies[22,23] involving healthy volunteers, as well as with studies from other Asian countries and most Middle Eastern countries, including Palestine and Turkey. Interestingly, a higher frequency of this variant has been reported by an Iranian study (3.9%)[24], this frequency was statistically different than our frequency ($p<0.05$). Additionally, two subsequent studies with larger sample sizes conducted in South Iran reported even lower frequencies of this variant allele, at 0.1% and 2.2%.[25,26] The frequency of the TPMT*2 allele in our study did not significantly differ from that observed in studies conducted in Europe and Africa. Additionally, the TPMT*3 A allele, which includes two genetic variations (G460A in exon 7 and A719G in exon 10), was not found in our study. *3A allele is commonly found in Caucasians.[27]

Many studies in White/Caucasian populations have reported significantly higher frequencies of this allele compared to our study. For example, the *3A allele has been observed at a frequency of 4.5% in British Caucasians and a similar rate has also been reported in German-Caucasians. Additionally, the frequency of the *3A allele was notably higher in some populations in the Americas compared to our findings. In contrast, the *3A allele frequency in Asian, African, and other Jordanian studies of healthy volunteers was similar to our results. TPMT*3B is a rare allele that is typically not found in most populations. The Mexican population has been reported to have a high prevalence of this variant allele, with its frequency reaching 2.3% among healthy volunteers.[28]

Research in the Jordanian population has shown the frequency of this allele to range from 0.0% to 1%. In European countries, the Spanish population exhibits a relatively higher frequency at 1.5%. However, significantly lower frequencies of this variant have been observed in the Russian (0/1990 alleles), British (0/2298 alleles), and German (0/2428 alleles) populations compared to our findings. Consequently, further prospective studies are needed to examine the relationship between TPMT polymorphisms and adverse reactions to 6-MP.

Conclusion

Study revealed a higher prevalence of significant polymorphisms in 6-MP metabolizing enzymes among Pakistani patients with ALL. These findings emphasize the critical role of TPMT genotyping in optimizing treatment strategies with 6-MP for ALL patients, enabling more tailored and effective therapy.

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