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Genetic Polymorphism of the Thiopurine S-Methyltransferase Gene: A Tertiary Care Setup Study

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ABSTRACT

Objective: To determine Thiopurine s-Methyl Transferase (TPMT) gene variants in patients reporting to a Tertiary Care Hospital in Pakistan.

Study Design: Cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi Pakistan, from May 2022 to Jun 2023.

Methodology: A total of 110 patients diagnosed with Inflammatory Bowel Disease and using were included in this study. DNA was extracted following the GeneJet genomic DNA purification method. Genotype for the respective mutation of extracted DNA for Thiopurine s-Methyl Transferase (TPMT) genes was determined using real-time Polymerase Chain Reaction (PCR) with the use of fluorescently labelled probes specifically on the principle of allelic discrimination. Gene and allele frequencies were calculated. Expected genotype frequencies were calculated using Hardy-Weinberg equation. Gene and allele frequencies were compared with expected frequencies using Chi square test to reveal any deviation from Hardy-Weinberg equilibrium.

Results: Among 110 patients enrolled in the study, 102 patients (92.7%) were found to have wild type alleles. Eight patients (7.3%) had variant (mutated) alleles of TPMT. Among these patients, 7(6.4%) had heterozygous allelic pattern, 5 had TPMT*3C while 2 had TPMT*3B. One patient (0.9%) was found homozygous TPMT*3C variant alleles. TPMT*2 variant was not found in any of the patient. Frequencies did not reveal any deviation from Hardy-Weinberg equilibrium (p>0.05).

Conclusion: TPMT*3C was the most common genetic variant found in heterozygous pattern. Gene and allele frequencies showed no deviation from the Hardy-Weinberg equilibrium.

Keywords: Inflammatory Bowel Disease, Real-Time PCR, Thiopurine Drugs, TPMT Gene, TPMT Variant Alleles, Wild Type Alleles.

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INTRODUCTION

Thiopurine s-Methyl Transferase (TPMT) is a cytoplasmic enzyme, required for inactivation of thiopurine drug.[1] Studies have revealed its autosomal codominant expression. Genetic polymorphism controlled, TPMT enzyme activity have been established at other cellular levels emphasizing its clinicopathological importance in humans.[2]

In recent years, there has been extensive use of thiopurine drugs in chemotherapy, as well as for multiple autoimmune disorders worldwide in treatment of inflammatory bowel diseases, acute lymphoblastic leukemia, rheumatoid arthritis, as immunosuppressants after organ transplantation, and other autoimmune disease.[3-5] Excessive accumulation of its metabolites, including thioinosine mono-phosphate, into cellular DNA can have severe cytotoxic effects especially on bone marrow. TPMT enzyme converts these metabolites into inactive molecules preventing cellular toxicity.[6]

TPMT enzyme activity is controlled by genetic polymorphism in humans resulting in different response to thiopurine drugs.[7] Therefore, drug efficacy and toxicity can be predicted by determining TPMT polymorphism in individuals of a specific ethnicity, race or region.[8] Various international studies have shown that patients requiring thiopurine drugs are at the risk of severe bone marrow suppression if they take conventional dosage of the drug without knowing the status of TPMT gene polymorphism.[9] Other International studies revealed "wild type" TPMT gene to be the most frequently encountered genotype requiring conventional dosage of thiopurine drugs. However, significant presence of TPMT variants was found in populations around the globe, requiring alternate drugs or tapered doses of thiopurines, necessitating screening protocol guidelines for TPMT genetic testing before starting these drugs to prevent toxicity.[10]

Very little is known regarding the presence of TPMT genetic variants in our population and we are

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exposing a significant number of patients to thiopurine induced toxicities. In this study, TPMT gene polymorphism was studied in patients who were candidates for thiopurine therapy reporting to a tertiary care hospital in Pakistan.

METHODOLOGY

This cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi Pakistan, from May 2022 to Jun 2023, after getting prior approval from Institutional Ethical Review Board (IRB certificate number-1458).

Inclusion Criteria: Adult patients of either gender who were diagnosed with inflammatory bowel disease and were candidates for thiopurine drug treatment, were included.

Exclusion Criteria: Patients with a previous history of drug reactions to thiopurine drugs and those who had features suggestive of hematopoietic suppression were excluded.

A total of 110 patients were recruited in this study using non-probability convenience sampling. WHO calculator was used to calculate the sample size for the study considering the 7.7% prevalence of Inflammatory Bowel Disease in Pakistan.[11] These patients were diagnosed at Gastroenterology OPD with inflammatory bowel disease and azathioprine was prescribed to them. Patients reported to Armed Forces Institute of Pathology for TPMT gene testing before start of thiopurine drugs. Informed consent was taken prior to the recruitment of patients.

All patients were interviewed and relevant history was taken, including demographic information on age, gender and ethnicity. From each patient, 5ml blood was collected in EDTA vacutainer using the universal precautions of blood sampling. DNA was extracted following the GeneJet genomic DNA purification method. Genotype for the respective mutation of extracted DNA for TPMT genes was determined using real-time PCR, with the use of fluorescently labelled probes specifically on the principle of allelic discrimination. A commercial kit by Generi Biotech was used. PCR cycler was set to following temperature profiles for amplification protocols: Initial denaturation was done at 95°C for 3 minutes, and Annealing and elongation was performed at 60°C for 20 seconds. Each sample was run separately on Qiagen Rotergene Q for 50 cycles. Real-time PCR was performed to determine the wild type alleles (TPMT2, TPMT3B, TPMT3C) and their genetic variants (TPMT*2, TPMT*3B, TPMT*3C) in participants of study. Positive and negative controls were run with each specimen analysis to ensure the quality control of results. Patients' privacy and confidentiality were ensured during the whole period of research.

Gene and allele frequencies were calculated and results were documented. Expected genotype frequencies were calculated using Hardy-Weinberg equation. Gene and allele frequencies were compared with expected frequencies using Chi-square test to reveal any deviation from Hardy-Weinberg equilibrium. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 110 individuals of inflammatory bowel disease were studied. Shapiro-Wilk test was applied to check the normalcy of data and data was found to be non-parametric (p<0.05). Median age of the patients came out to be 44 years (IQR 34-49.25 years). Sixty-seven patients (60.9%) were male whereas 43(39.1%) were female. Variant alleles were found more in females (11.6%) as compare to males (4.5%). In this study 76 patients (69.1%) belonged to Punjab, Pakistan and 1 patient (0.9%) represented Baluchistan, Pakistan as shown in Table-I. There was no significant association between ethnicity and genetic mutation (p>0.05).

Table-I: Ethnicity of Patient who were TPMT Genotyped (n=110)

Ethnicity	Total n(%)	Mutation		p-value
		Present n(%)	Absent n(%)	
Punjab	76(69.1%)	5(62.5%)	71(69.6%)	0.599
Sindh	10(9.1%)	0(0.0%)	10(9.8%)	
Khyber-Pakhtunkhwa	18(16.4%)	3(37.5%)	15(14.7%)	
Baluchistan	1(0.9%)	0(0.0%)	1(1.0%)	
Gilgit Baltistan	2(1.8%)	0(0.0%)	2(2.0%)	
Azad Kashmir	3(2.7%)	0(0.0%)	3(2.9%)	

Three different alleles and genes (TPMT2, 3B and 3C) already studied internationally and commonly observed in different populations of the world, were determined along with their polymorphism at loci c.719A>G, c.460G>A and c.238G>C. Among the total participants enrolled in this study, 102 patients (92.7%) had the "wild type" genotype and 8 patients (7.3%) had mutant genotype. Out of these variants, 5(4.5%) were heterozygous for TPMT*3C, 2(1.82%) were heterozygous for TPMT*3B and 1(0.9%) had TPMT*3C

homozygous as well as TPMT*3B heterozygous pattern as shown in Table-II.

Table-II: Frequencies of different TPMT Genotypes in total Patients (n=110)

Genotype Distribution	Frequency & Percentage of Genotype n (%)
Wild Type (Homozygous)	102(92.65)
Mutant Type (Heterozygous)	7(6.45)
Mutant Type (Homozygous)	1(0.9)

Similarly, allele frequencies were also calculated. Two hundred and eleven alleles appeared to be wild type and most numerous. Calculated frequency of alleles is shown in the Table-III.

Table-III: Frequencies of Different TPMT Alleles

Allelic Type	n(%)
Wild Type TPMT	211(95.91)
TPMT 2*	0(0)
TPMT 3B*	2(0.90)
TPMT 3C*	7(3.19)

TPMT*2 variant was not found in any of the individuals in this study. An insignificant *p*-value (0.486) was observed when the calculated genotype frequency was compared to expected frequencies. Hence, these frequencies did not reveal any deviation from Hardy-Weinberg equilibrium.

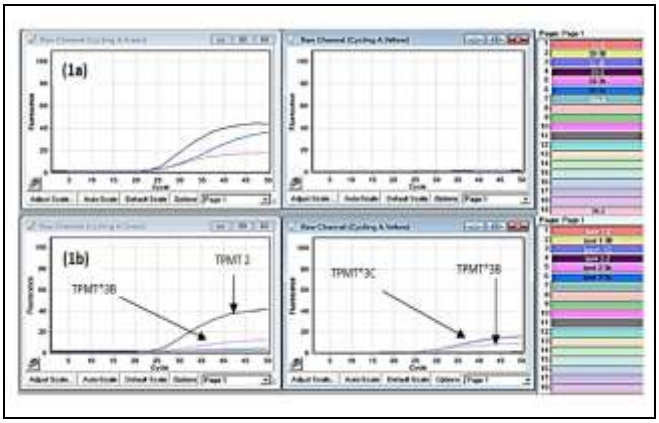

Figure-1: Different types of Alleles

Figure-1 shows different types of alleles. Wild type alleles are labeled with Flouresceinamide (FAM) as shown on green channel whereas mutant alleles are labeled with 5′-Dichloro-dimethoxy-flourescein (JOE) as shown on yellow channel. Figure (1a) shows specimen with wild type TMPT2, 3B, and 3C. Figure (1b) shows specimen with homozygous wild type TPMT 2 (Purple), heterozygous TPMT*3B (Pink) and homozygous TMPT*3C (Blue).

DISCUSSION

Thiopurine s-methyl transferase (TPMT) enzyme is required for conversion of thiopurine drugs into their inactive metabolites. These thiopurine drugs are now frequently being used in our part of the world as immunosuppressive therapy for various diseases like inflammatory bowel diseases, rheumatoid arthritis, vasculitis, leukemia, autoimmune vasculitis and bone marrow transplantation.[12] International literature reveals that three most commonly found TPMT gene variants have direct effect on the thiopurine drug's metabolism by altering the TPMT enzyme levels, emphasizing its role in pharmacogenomics. Patients with lower inherent levels of this enzyme are vulnerable to severe hematopoietic suppression, if not identified and had either dose adjustment or alternate drug therapy selected.[13,14]

In our study, "wild type" alleles were detected in 92.7% patients whereas mutant alleles were present in 7.30% among total patients. TPMT*3C was the predominant mutant allele found in this study (5.46%) whereas frequency of TPMT*3B was relatively low (1.82%).

The frequency of TPMT variants observed in our study is similar to those determined in studies conducted in North American (6-11%), British (5.3), Nigerian (9.4%), Swedish (9%), French (8.1%), Brazilian (7%) and Latvian (6.1%) individuals.[15-19] Collie-Duguid *et al.,* conducted a study on 490 healthy Chinese, South-west Asian and Caucasian individuals.[15] Among 490 participants, 218 were female and 272 were male. All participants included these groups were unrelated. Results revealed 10.1% TPMT variant genotype in Caucasians (with TPMT*3A predominance), 2% in south-west Asians (with TPMT*3A predominance) and 4.7% in Chinese (with TPMT*3C predominance). The Caucasian group had the highest frequency of TPMT gene variants as compared to other groups. Similarly, the frequency of TPMT variants was studied by Mora *et al.,* in 150 healthy individuals from Venezuela.[20] It revealed 10(6.7%) healthy residents with TPMT variants detected by PCR-restriction fragment length polymorphism assay. Four percent of patients were TPMT*3A, 2% TPMT*3C and 0.7% TPMT*2. In contrast, various studies have found that the frequency of TPMT variants was slightly low in Egyptian (3%), Turkish (2.7%), Indian (1.7%), Indonesian (1.9%) and Libyan (1.63) people.[21-24] Murugesan *et al.,* revealed that among 326 participants

of their study, 317 participants (97.24%) had "wild type" genotype while 9(2.74%) had TPMT variants. Five participants (1.53%) were TPMT*3C heterozygous, 2(0.61%) were TPMT*3B heterozygous and 2(0.61%) were TPMT*2 heterozygous.[25]

Based on our findings, it is recommended that TPMT genetic testing may be incorporated as screening test in our national guidelines, for the patients who are candidates of thiopurine drugs. This will help us to avoid significant number of patients from developing disastrous hematopoietic adverse reactions while using thiopurine drug treatment.

LIMITATION OF THE STUDY

This study is a single center tertiary care setup study and common variants of TPMT alleles and their genotypes were tested.

CONCLUSION

TPMT*3C was the most common genetic variant found in heterozygous pattern. Gene and allele frequencies showed no deviation from the Hardy-Weinberg equilibrium.

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Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NUR & ZHH: Conception, study design, drafting the manuscript, approval of the final version to be published.

AH & HGK: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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