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RESEARCH ARTICLE
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PROPORTION OF TPMT AND NUDT 15 GENETIC POLYMORPHISM FOR AZATHIOPRINE METABOLISM

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Abstract

Background: This study explores the prevalence of genetic polymorphisms in the TPMT and NUDT15 genes and their association with Azathioprine-induced leucopenia in South Asia, focusing on Kerala. Azathioprine, a commonly used immunosuppressive medication, is crucial in managing Inflammatory Bowel Disease (IBD) and Autoimmune Hepatitis. This research seeks to determine the impact of these polymorphisms and their potential role in individualized treatment strategies.

Methods: Conducted as a prospective observational study, the research involves patients diagnosed with IBD and Autoimmune Hepatitis. Genetic analysis is employed to estimate the prevalence of TPMT and NUDT15 genetic polymorphisms. The study underscores the necessity of genetic assessments to inform treatment decisions and improve safety by minimizing severe adverse reactions.

Results: we observed genetic variation in NUDT 15 genotyping, with allele frequencies predominantly showing the 1* allele (90.7%) and a heterozygous allele combination of 1*/3* (9.3%), evidenced by the c.415C> T:p,R139C variant. Additionally, all participants demonstrated the genetic polymorphism TPMT*1/*1 (wild-type), indicating normal enzymatic activity. The study provides insights into patient demographics, clinical history, symptoms, severity, and treatment modalities.

Discussion emphasizes the rising incidence of IBD and Autoimmune Hepatitis in South Asia, particularly India, and the cost-effective significance of Azathioprine in managing these conditions. Genetic variations, particularly in NUDT15 gene variants, are identified as predictors of Azathioprine-induced leucopenia. The study underscores the potential of NUDT15 genotyping for personalized dosing, aligning with recommendations from the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines.

Conclusion: In conclusion, this research underscores the importance of understanding genetic variations to optimize Azathioprine therapy. By incorporating genetic insights, notably NUDT15 genotyping, clinicians can enhance treatment decisions, patient safety, and overall care quality. Routine CBC monitoring is recommended as a cost-effective strategy for Azathioprine treatment, enabling proactive management of adverse effects. This study contributes valuable insights for informed therapeutic strategies and improved treatment outcomes in the South Asian population.

Keywords: TPMT, NUDT, Inflammatory Bowel Disease, Azathioprine.

INTRODUCTION

Genetic variations within the thiopurine methyltransferase (TPMT) and Nudix hydrolase-15 (NUDT15) genes have emerged as primary contributors to the development of leucopenia induced by Azathioprine, a commonly employed immunosuppressive medication[1]. However, the precise impact of these polymorphisms within the South Asian population, particularly in the context of Kerala, remains to be definitively established.

In the field of medical gastroenterology, Azathioprine finds prominent application in the management of conditions such as Inflammatory Bowel Disease (IBD) and Autoimmune Hepatitis. Nonetheless, the occurrence of adverse effects, notably leucopenia, pancreatitis, and cholestatic hepatitis, has underscored the importance of individualized treatment approaches[2].

Given the genetic diversity among populations, it becomes imperative to discern the distribution and prevalence of TPMT and NUDT15 polymorphisms in the Kerala population before initiating Azathioprine therapy. This preemptive genetic assessment would facilitate informed therapeutic decisions, minimize the risk of severe adverse reactions, and optimize treatment outcomes.

In summary, variations in TPMT and NUDT15 genes have been identified as pivotal players in Azathioprine-induced leucopenia. The ramifications of these polymorphisms within the South Asian populace, particularly in Kerala, necessitate further investigation. In the realm of medical gastroenterology, Azathioprine's utility in addressing conditions like IBD and Autoimmune Hepatitis is noteworthy, albeit accompanied by potential adverse effects. Hence, a thorough understanding of TPMT and NUDT15 genetic makeup in the Kerala population is imperative to enhance the safety and efficacy of Azathioprine therapy.

MATERIALS AND METHODS

In this prospective observational study, the objective was to estimate the prevalence of TPMT and NUDT15 genetic polymorphisms in patients diagnosed with Inflammatory Bowel Disease (IBD) and Autoimmune Hepatitis prior to the initiation of Azathioprine therapy. The research question addressed was the prevalence of TPMT and NUDT15 genetic polymorphisms in patients with IBD and Autoimmune Hepatitis. The study, conducted over 12 months at Government Medical College in Kottayam, employed a prospective observational design within the medical gastroenterology outpatient and inpatient departments. It enrolled adult patients diagnosed with IBD or Autoimmune Hepatitis, intending to initiate Azathioprine therapy, after obtaining informed consent. Sample size determination, referencing a study by Narinder Grover et al., resulted in a calculated sample size of 270, based on a 27% prevalence of leucopenia, utilizing the formula 4pq/d^2, where p represents prevalence, q is its complement, and d denotes desired precision.

Study Tools and Data Collection
Data were collected using a semi-structured questionnaire and laboratory investigation reports. Demographic and clinical details of the patients, including underlying diagnosis, recent steroid use, aminosalicylate use, Montreal classification of IBD, and simplified scoring system for autoimmune hepatitis, were recorded.

Inclusion Criteria
All patients aged 18 and above diagnosed with IBD or Autoimmune Hepatitis who were planning to start Azathioprine were included after obtaining informed consent.

Exclusion Criteria
Patients with neutropenia (absolute neutrophil count less than 1500/mm^3) and those who lacked informed consent were excluded from the study.

Study Procedure
Following IRB clearance and patient consent, the study involved consecutive patients admitted to the medical gastroenterology department with IBD or Autoimmune Hepatitis (prior to Azathioprine initiation) over a 12-month period. Relevant patient information, including demographic and clinical data, was documented. Azathioprine therapy (1 mg/kg for IBD, 50 mg/day for autoimmune hepatitis) was initiated based on TPMT and NUDT15 genetic polymorphism reports.

Genetic Analysis
Blood samples (3 ml) were collected through venipuncture in EDTA vials. Genetic analysis for Azathioprine metabolism-related polymorphisms (TPMT and NUDT15) was performed. TPMT gene mutations causing deficiency (c.238 G>C, c.460 G>A, and c.719 A>G) were determined using Amplification Refractory Mutation System (ARMS) PCR and PCR amplification with restriction enzyme digestion (PCR–RFLP). NUDT15 gene mutation analysis was conducted using PCR-RFLP to detect mutations.

Follow-up and Data Analysis
Patients with normal genetic polymorphism were followed up for 6 months, specifically to monitor leucopenia and idiosyncratic reactions. Patients with abnormal genetic polymorphism were considered for alternative treatment options. The study also accounted for the possibility of intolerance to Azathioprine or loss of follow-up in the long run.

Ethical Consideration
The study outlined above was conducted within this institution subsequent to securing ethical clearance from the Institutional Review Board (IRB) of Government Medical College, Kottayam, specifically from the Department of Medical Gastroenterology. Informed consent was acquired from all patients participating in the study.The costs for TPMT and NUDT15 analyses were covered under KASP insurance.

Statistical Analysis
Quantitative Variables were expressed as mean and standard deviation. Qualitative variable were expressed as frequency and percentage. Association between categorical variables was analysed by chi-square test. Comparison of continuous variables between two group was analysed by independent sample t test. A p- values <0.05 was considered as statistically significant. Data was entered in Microsoft excel and Data analysis was performed using SPSS ver 24.

RESULTS

Demographics and Medical History
- The study included a total of 270 participants.

- The mean age of the participants was 39.2 years, with a range of 14 to 60 years. (Table 1)
- The majority of participants are female (56.3%) and have a past history of illness (52.6%).
- The most common past illnesses included hypothyroidism (36.3%) and diabetes mellitus (21.1%). (Table. 2)
- A significant portion of male participants had not smoked (82.2%) or consumed alcohol (88.5%). none of the female subjects were smokers (graph 1.0, graph 2.0)

Primary Diagnosis

- The primary diagnoses included autoimmune hepatitis (37.4%), IBD UC (27.8%), and IBD/CD (34.8%). (graph 3.0)
- The duration of the disease varies, with the majority of participants had a disease duration of less than 3 months (57.4%). (Table 3)

Symptoms and Severity

- Symptoms experienced by participants after Azathioprine use with normal allele for TPMT includes Leukopenia with neutropenia -10(3.7%), fever: 20 cases (7.4), Nausea: 44 cases (16.3%), Skin rash: 0 cases (0.0%), Alopecia: 97 cases (35.9%), Headache: 14 cases (5.2%), Pancreatitis: 3 cases (1.2%)
- The severity of the IBD varies, with the majority of participants had mild to moderate severity.
- Most common extra articular manifestation noticed was pauciarticular arthropathy (20.6%) (graph 4)

Genetic Polymorphism

- All participants exhibit the genetic polymorphism TPMT*1/*1 (wild-type) with normal enzymatic activity (table 4)

Medication and Treatment

- A significant proportion of IBD patients are using oral mesalamine (88.1%) and steroids (79%).
- Biological usage includes adalimumab (8.5%), infliximab (10.9%), and tofacitinib (3.0%).

Genetic Variation (NUDT 15 Genotyping)

- NUDT 15 genotyping shows variations with allele frequencies of 1* allele (genetic variant -none) (90.7%) and 1*/3* (9.3%-heterozygous allele). c.415C>T: p,R139C (table 4)

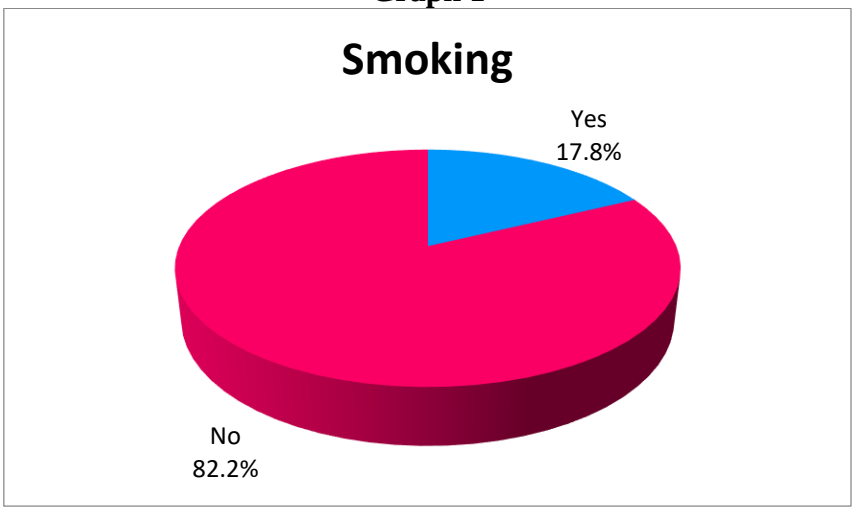
Age in years	Frequency	Percent
<20	31	11.5
21-30	42	15.6
31-40	68	25.2
41-50	69	25.6
>50	60	22.2
Total	270	100
Table 1		

Past history of illness	Frequency	Percent
DM	57	21.1
HTN	20	7.4
Hypothyroidism	98	36.3
CLD	5	1.9
CAD	4	1.5
Asthma	10	3.7
Hep B	5	1.9
Table 2		

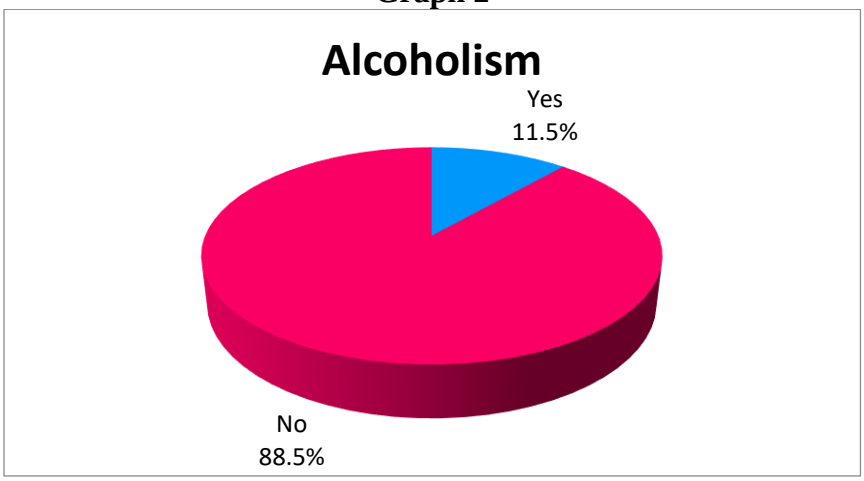
TOTAL DURATION OF DISEASE	Frequency	Percent
<3 months	155	57.4
3-6 months	57	21.1
6-12 months	36	13.3
>12 months	22	8.1
Total	270	100

Table 3

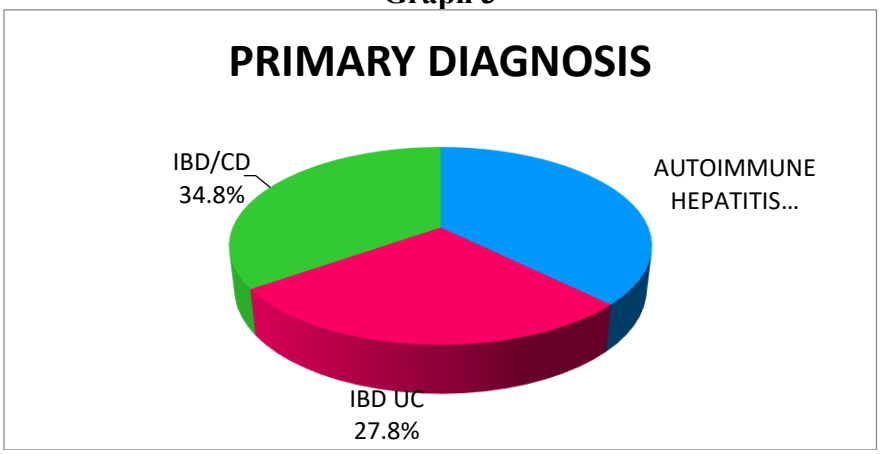
Graph 1



Graph 2



Graph 3



GENETIC POLYMORHISM TPMT	Frequency	Percent
*1/*1The wild-type TPMT*1/TPMT*1	270	100
Table 4		

NUDT15 GENOTYPING	Frequency	Percent
1	245	90.7
3	25	9.3
Total	270	100
Table 5		

DISCUSSION

In recent years, the Southeast Asian region, including India, has witnessed a notable rise in the incidence of Inflammatory Bowel Disease (IBD) and Autoimmune Hepatitis. Azathioprine has emerged as a commonly used second-line steroid sparing agent, primarily due to its cost-effectiveness in comparison to biological treatments[3]. This study aims to explore the growing prevalence of IBD and Autoimmune Hepatitis in kerala, the prominence of Azathioprine, and the role of Nudix Hydrolase 15 (NUDT15) gene variants in predicting adverse reactions to this drug.

This study sheds light on the prevalence of genetic polymorphisms in TPMT and NUDT15 genes and their association with Azathioprine-induced leucopenia in the Indian population, with a specific focus on Kerala. The findings corroborate prior research, indicating a predominance of TPMT*1/*1 (wild-type) polymorphism among participants, aligning with studies emphasizing its role in Azathioprine metabolism. Notably, the study uncovers a significant proportion of NUDT15 gene variations, particularly the c.415C>T:p, R139C variant, highlighting its relevance in predicting adverse reactions to Azathioprine. These results underscore the importance of genetic assessments before initiating Azathioprine therapy, advocating for personalized treatment strategies to optimize patient safety and outcomes.

The discussion emphasizes the rising incidence of IBD and Autoimmune Hepatitis in kerala, paralleling global trends, while emphasizing the cost-effective significance of Azathioprine as a second-line therapy compared to biologics. Despite the study's strengths, including a substantial sample size and detailed genetic analysis, certain limitations exist. The lack of long-term follow-up data limits the assessment of treatment outcomes and adverse reactions over time. Additionally, while the study provides valuable insights into genetic polymorphisms, it lacks direct comparison with prior studies in the South Asian context, hindering a comprehensive understanding of regional variations and genetic predispositions. Addressing these limitations through further longitudinal research and comparative analysis with broader population cohorts would enhance the generalizability and clinical relevance of the findings.

Surging Incidence of IBD and Autoimmune Hepatitis

The escalating incidence of IBD and Autoimmune Hepatitis in Southeast Asia, particularly in India, has raised concerns. The reasons for this surge are multifaceted, including changes in lifestyle, environmental factors, and possibly genetic predisposition. These conditions pose significant healthcare challenges, prompting researchers and clinicians to explore effective treatment options.

Azathioprine: A Cost-Effective Steroid Sparing Agent

In the arsenal of IBD and Autoimmune Hepatitis treatments, Azathioprine has garnered attention due to its economic viability as a second-line therapy compared to pricier biological drugs. Azathioprine's mechanism of action involves inhibiting small GTPase, leading to reduced protein synthesis in lymphocytes. This results in apoptosis and decreased proliferation of T-lymphocytes, thus exhibiting its immunosuppressive properties in these conditions[4,5,6,7].

NUDT15 Variants and Adverse Reactions

Azathioprine is a prodrug that transforms into 6-mercaptopurine (6-MP) in the body. The metabolism of 6-MP is complex and involves various intermediates, including 6-thioguanine (6-TG), a metabolite linked to both the drug's therapeutic effects and its toxicity[8,9]. Adverse effects associated with Azathioprine are classified as idiosyncratic and include flu-like syndrome, gastrointestinal intolerance, acute pancreatitis, and cytopenias[10,11].

Recent research has spotlighted the significance of NUDT15 gene variants in predicting adverse reactions to Azathioprine. East Asian populations, including India, exhibit varying prevalence of these variants[12,13]. In particular, the NUDT15 variant p.Arg139Cys has been linked to early leucopenia induced by Azathioprine. Indian studies have reported that NUDT15 variant genotyping holds greater predictive accuracy for leucopenia than TPMT genotyping. Furthermore, NUDT15 variants have demonstrated a positive gene dose effect, with increased risk of severe leucopenia correlated to the number of risk alleles[14,15,16].

Clinical Implications and Future Prospects

The implications of NUDT15 variants extend beyond predicting leucopenia risk. These genetic markers have the potential to guide personalized dosing strategies for thiopurine medications, aiding in treatment optimization and minimizing adverse effects. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines reflect this growing understanding by recommending dosing adjustments based on NUDT15 genotypes[17].

While current studies underscore the importance of NUDT15 variants, further research is warranted, particularly in the South Asian context, to delineate the implications of these variants comprehensively. Addressing questions of effectiveness, applicability, and potential ethnicity-based differences will provide a more comprehensive understanding of the role of NUDT15 genotyping in clinical practice.

CONCLUSION

The increasing prevalence of Inflammatory Bowel Disease (IBD) and Autoimmune Hepatitis in South Asia, including India, has elevated the importance of Azathioprine as a cost-effective second-line treatment. Azathioprine's mechanism of action, inhibiting small GTPase and suppressing T-lymphocyte proliferation, makes it a valuable tool in managing these conditions.

However, its benefits are accompanied by potential adverse effects, including leucopenia. Genetic variations, especially in the Nudix Hydrolase 15 (NUDT15) gene, play a pivotal role in Azathioprine-induced leucopenia. Beyond prediction, NUDT15 variants offer personalized dosing potential. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines endorse NUDT15 genotyping for dose adjustments, optimizing treatment and minimizing adverse effects. Further research, particularly in regions like Kerala, is needed to understand NUDT15's implications fully. Amid rising IBD and Autoimmune Hepatitis cases, Azathioprine remains a crucial option. Incorporating genetic insights, especially NUDT15 genotyping, enhances treatment decisions, patient safety, and overall care quality. As a cost-effective strategy, routine CBC monitoring is preferred over genetic polymorphism testing for treatment with Azathioprine.

REFERENCES

1. Grover, N., Bhatia, P., Kumar, A. et al. TPMT and NUDT15 polymorphisms in thiopurine induced leucopenia in inflammatory bowel disease: a prospective study from India. BMC Gastroenterol 21, 327 (2021)
2. Yates CR, Krynetski EY, Loennechen T, et al. Molecular diagnosis of thiopurine S-methyltransferase defciency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med. 1997;126(8):608–14.
3. Sood A, Midha V, Sood N, Bansal M. Long term results of use of azathioprine in patients with ulcerative colitis in India. World J Gastroenterol. 2006 Dec 7;12(45):7332-6.

4. Fraser AG, Orchard TR, Jewell DP. The efcacy of azathioprine for the treatment of infammatory bowel disease: a 30 year review. Gut. 2002;50(4):485–9.
5. Vasudevan A, Parthasarathy N, Con D, Nicolaides S, Apostolov R, et al. Thiopurines vs methotrexate: comparing tolerability and discontinuation rates in the treatment of infammatory bowel disease. Aliment Pharmacol Ther. 2020;52(7):1174–84.
6. Matsuoka K. NUDT15 gene variants and thiopurine-induced leukopenia in patients with infammatory bowel disease. Intest Res. 2020;18(3):275–81.
7. Seinen ML, van Nieuw Amerongen GP, de Boer NK, van Bodegraven AA. Rac attack: modulation of the small GTPase rac in infammatory bowel disease and thiopurine therapy. Mol Diagn Ther. 2016;20(6):551–7.
8. Elion GB. The purine path to chemotherapy. Science. 1989;244(4900):41–7
9. van Gennep S, Konté K, Meijer B, Heymans MW, D' Haens GR, Löwenberg M, de Boer NKH. Systematic review with meta-analysis: risk factors for thiopurine-induced leukopenia in IBD. Aliment Pharmacol Ther. 2019;50(5):484–506.
10. Van Scoik KG, Johnson CA, Porter WR. The pharmacology and metabolism of the thiopurine drugs 6-mercaptopurine and azathioprine. Drug Metab Rev. 1985;16(1–2):157–74.
11. Nguyen CM, Mendes MA, Ma JD. Thiopurine methyltransferase (TPMT) genotyping to predict myelosuppression risk. PLoS Curr. 2011;3:RRN1236
12. Yang S-K, Hong M, Baek J, et al. A common missense variant in nudt15 confers susceptibility to thiopurine-induced leukopenia.Nat Genet. 2014;46:1017-1020.
13. Banerjee R, Ravikanth VV, Pal P, Bale G, Avanthi US, Goren I, Girish BG, Mitnala S, Reddy DN. NUDT15 C415T variant compared with TPMT genotyping in predicting azathioprine-induced leucopenia: prospective analysis of 1014 inflammatory bowel disease patients in India. Aliment Pharmacol Ther. 2020 Dec;52(11-12):1683-1694.
14. Takatsu N, Matsui T, Murakami Y, et al. Adverse reactions to azathioprine cannot be predicted by thiopurine S-methyltransferase genotype in Japanese patients with inflammatory bowel disease. J Gastroenterol Hepatol. 2009;24:1258-1264.
15. Chao K, Wang X, Cao Q, et al. Combined detection of NUDT15 variants could highly predict thiopurine-induced leukopenia in Chinese patients with inflammatory bowel disease: a multicenter analysis. Inflamm Bowel Dis. 2017;23:1592-1599.
16. Jena A, Jha DK, Kumar-M P, Kasudhan KS, Kumar A, Sarwal D, Mishra S, Singh AK, Bhatia P, Patil A, Sharma V. Prevalence of polymorphisms in thiopurine metabolism and association with adverse outcomes: a South Asian region-specifc systematic review and meta-analysis. Expert Rev Clin Pharmacol. 2021;14(4):491–501
17. Relling MV, Schwab M, Whirl-Carrillo M, et al. Clinical pharmacogenetics implementation consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. ClinPharmacol Ther. 2019;105:1095-1105.