www.jhas-bhs.com

Journal of Hematology and Allied Sciences

Case Series

Severe bone marrow aplasia following azathioprine therapy in patients with NUDT15 deficiency: A case series

Navneet Mishra[1], Naman Bansal[1], Vandana Arya[2], Jyoti Kotwal[2], Nitin Gupta[1]

Departments of [1]Clinical Hematology and Bone Marrow Transplant, [2]Hematology, Sir Ganga Ram Hospital, New Delhi, India.

***Corresponding author:**
Nitin Gupta,
Department of Clinical Hematology and Bone Marrow Transplant, Sir Ganga Ram Hospital, New Delhi, India.

docnitingupta@gmail.com

Received: 22 September 2024
Accepted: 06 January 2025
Published: 13 February 2025

DOI
10.25259/JHAS_51_2024

Quick Response Code:

Supplementary Link
https://dx.doi.org/10.25259/JHAS_51_2024

ABSTRACT

Azathioprine is widely used in various autoimmune disorders, hematological malignancies, inflammatory bowel disease, skin disorders, and in post renal transplants as an immunosuppressant. Myelosuppression is a well-known adverse drug reaction related to thiopurines including azathioprine, 6-mercaptopurine, and thioguanine. Thiopurine S-methyltransferase (TPMT) and Nudix hydrolase 15 (*NUDT15*) are two critical enzymes involved in metabolism of azathioprine and other thiopurines. Genetic polymorphism affects enzyme activity, and thus metabolism of these drugs (Azathioprine, 6-mercatopurine, and thiogunine). People with enzyme deficiency are at increased risk of prolonged and severe myelosuppression following treatment with azathioprine and other thiopurine drugs. Loss-of-function alleles in the *NUDT15* gene are reported to be common than TPMT in south Asian population. Here, we are reporting six cases of *NDUT15* polymorphism that was admitted in our institute. Retrospective data were collected from the January 2024 to June 2024 of patients who were admitted at our institute. We also reviewed PubMed data base for literature on NUDT15 polymorphism in south Asian population. In conclusion, NUDT15 polymorphism is more prevalent in south Asian population, and thus, testing for NUDT15 polymorphism could help to individualize thiopurine therapy better in the South Asian population and should be done in the patients who experience severe myelosuppression following introduction of azathioprine and other thiopurines.

Keywords: Azathioprine therapy, Bone marrow aplasia, Nudix hydrolase 15, Thioguanine nucleotides, Thiopurine S-methyltransferase

INTRODUCTION

Azathioprine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites, which further metabolized and inactivated by the enzyme thiopurine methyltransferase (TPMT) and the enzyme nucleoside diphosphate-linked moiety X-type motif Nudix hydrolase 15 (NUDT15) belongs to family Nudix hydrolase15.[1] Thiopurine methyltransferase (TPMT) inactivates azathioprine (AZA) resulting in less parent drug available to form 6-TGNs while Nudix hydrolase 15 is involved in the metabolism of azathioprine and other thiopurines, as it catalyzes the conversion of active metabolites thioguanine diphosphate (TdGTP) to less toxic metabolites thioguanine monophosphate (TdGMP) and in doing so, prevents the incorporation of toxic metabolites into deoxyribonucleic acid (DNA) [Supplementary Figure S1].[1] Genetic polymorphism affects enzyme activity, and thus metabolism of azathioprine. People with enzyme deficiency due to TPMT and NUDT15 polymorphism are at increased risk of prolonged and severe

life-threatening myelosuppression following treatment with azathioprine and other thiopurine drugs, and may require long in-hospital stay and supportive care. TPMT polymorphism is well known cause of myelosuppression following azathioprine therapy, but TPMT polymorphism is less commonly reported in south Asian population,[2] including in our case series all patients that were included in study were negative for TPMT polymorphism and are positive for NUDT15 polymorphism. Loss-of-function allele (Polymorphism: c.415C>T) in nucleoside diphosphate-linked moiety X motif 15 NUDT15 has been recognized to contribute to thiopurine tolerance in patients of Asian ancestry.[3] This variant frequency has been described commonly in East Asians and Hispanics, but rarer in the Europeans and could be the reason for myelosuppression following thiopurines drugs as reported frequency of TPMT polymorphism lower.[2] While testing for TPMT mutation in patients developing myelosuppression is well known and available, NUDT15 testing is not widely available. Routine testing for NUDT15 is not recommended before starting thiopurine drugs but it should be done in patients who develop severe myelosuppression following these drugs.[1] Here, we report our experience of severe myelosuppression with bone marrow aplasia following azathioprine therapy in six patients who were found to have NUDT15C415T variant and TPMT wild type.

CASE SERIES

Material and methods

Retrospective data were collected from records of clinical hematology department in our institute from January 2024 to June 2024 of patients who were admitted with the severe pancytopenia following azathioprine therapy. Demographic, clinical, treatment record, and laboratory data were collected and recorded [Table 1]. TPMT polymorphism testing was done by reverse dot blot assay method and NUDT15 polymorphism was done by allele specific polymerase chain reaction (PCR) method.

RESULTS

This is a retrospective case series of six patients, who developed severe myelosuppression while on AZA therapy. Out of all six patients, three patients were taking azathioprine for inflammatory bowel disease, two patients were taking drug for atopic dermatitis, and one patient was post renal transplant. Mean duration between introduction of azathioprine and severe pancytopenia was 26 days. Bone marrow aspiration and biopsy was done in three patients who revealed overall cellularity around 15–20%. In all six patients, TPMT was normal (Wild type*1/*1), while five patients were homozygous for NUDT15C415T mutation and one patient was heterozygous for NUDT15 polymorphism. All the patients received supportive blood component transfusion, colony-stimulating factor in view of severe neutropenia long with empirical antimicrobials. Patients were admitted till blood counts recovery and discharged after mean 12.5 days of in-hospital stay, recovery of blood counts was defined as ANC >500/microlitre for 3 days and platelets counts of >20000/microlitre for 3 days .

DISCUSSION

Azathioprine is a prodrug that must be metabolized to TGNs, the major active metabolites. The active metabolites

Table 1: Demographic, clinical, and laboratory data.

Case number	Age	Sex	Primary disease	Duration between azathioprine therapy and onset of severe pancytopenia	CBC at the of admission hemoglobin (gm/L)/TLC (10^9/L)/ANC (10^9/L)/platelet counts (10^9/L)/reticulocyte count (10^9/L)	Bone marrow biopsy cellularity	NUDT15 variant	Time to recovery of cell counts
1	18	Female	Ulcerative colitis	27 days	53/1.3/0.13/70/17	15–20%	NUDT15C415T Homozygous	15 days
2	32	Female	Ulcerative colitis	30 days	93/1.12/0.11/17/27	Not done	NUDT15C415T Homozygous	10 days
3	23	Male	Atopic dermatitis	21 days	76 /0.1/0/23/25	10–15%	NUDT15C415T Homozygous	17 days
4	51	Male	Atopic dermatitis	32 days	74/0.7/0.12/43/16	15–20%	NUDT15C415T Homozygous	14 days
5	36	Female	Post renal transplant	25 days	68/1.04/0.79/90/23	Not done	NUDT15C415T Heterozygous	11 days
6	61	Male	Crohns disease	18 days	89/0.06/0.01/5/17	Not done	NUDT15C415T Homozygous	8 days

CBC: Complete blood counts, TLC: Total leukocyte counts, ANC: Absolute neutrophil counts, NUDT15: Nudix hydrolase 15

are metabolized and inactivated by the enzyme thiopurine methyl transferase (TPMT) and the enzyme nucleoside diphosphate-linked moiety X-type motif (NUDT15).[1] Azathioprine toxicity manifests as hepatotoxicity, alopecia, and bone marrow suppression that may lead to severe neutropenia rendering patient to increased risk of life-threatening infections, severe anemia, and bleeding.[1] Myelosuppression is dose-dependent and correlates with the level of active metabolite TGN, and hepatotoxicity is related to 6-Methyl mercaptopurine (6-MMP).[3] Asians are found to tolerate AZA poorly and are more susceptible to toxicity, but paradoxically the common genetic polymorphisms in TPMT gene with reduced enzyme activity (TPMT×2, ×3) are extremely rare in Asians compared to the frequency reported in the European population.[2] Polymorphisms in thiopurine methyl transferase (TPMT) and NUDT15 have been implicated as the predominant cause of thiopurine induced leukopenia in the Western countries and East Asia, respectively.[4] Jena *et al*. in meta-analysis of 26 studies found, high prevalence of NUDT15 (16.5%) compared to TPMT polymorphisms (4.57%). In patients with adverse effects following thiopurine therapy, the pooled prevalence of NUDT15 and TPMT polymorphism was 49.51% and 9.47%, respectively.[5]

Gene NUDT15 encodes an enzyme that belongs to the Nudix hydrolase superfamily. NUDT15 is directly involved in the metabolism of thiopurines, as it catalyzes the conversion of active metabolites (TdGTP) to less toxic metabolites (TdGMP) and in doing so, prevents the incorporation of toxic metabolites into DNA.[1] Individual with reduced or absent NUDT15 activity are prone to severe adverse event related to azathioprine and other thiopurines. NUDT15C415T (p.R139C) is the first reported variant with absent enzyme activity and it is associated with both NUDT15 × 2 and NUDT15 × 3 genotype.[6] Similar to TPMT, PharmaVar consortium has catalogued 26 variants of NUDT15 and NUDT15 × 2 and NUDT15 × 3 genotypes are most commonly reported variants. The same genetic variant was also linked with 6-mercaptopurine (6-MP)-related myelosuppression in patients of East Asian ancestry with acute lymphoblastic leukemia.[2] In a retrospective study from south India by Babu *et al*. on 17 adult patients with acute leukemia, the prevalence of NUDT15 polymorphism using allele specific real-time PCR was 36%. They also studied impact of omission of 6-MP from the treatment protocols of the patient who were positive for NUDT15 mutation and found that less treatment discontinuation and interruption was noted in these patients.[7]

In this case series, the five patients were homozygous for NUDT15 polymorphism and one patient was heterozygous and it can be seen that, cytopenias occurred earlier (within 30 days) and it was more severe. AZA was stopped in all of them and was never re-challenged. All patients required longer in hospital stay and supportive treatment. Surprisingly, all patients were normal for TPMT mutation, so it can be stated that NUDT15 polymorphism is more prevalent in north India also and testing is also cost effective. Furthermore, polymorphisms in NUDT15 can explain and predict the occurrence and severity of myelosuppression seen in Indian patients.

To the best of our knowledge, no study has been published from north India in patients with severe pancytopenia and bone marrow hypoplasia following azathioprine therapy in NUDT15 mutated patients.

CONCLUSION

This report indicates that NUDT15 polymorphism is an important determinant of myelosuppression related to the intake of azathioprine. As compared to TPMT polymorphism, NUDT15 polymorphism is more frequent in north Indian population, and thus, testing for the common variants of NUDT15 associated with thiopurines toxicity is necessary in patients who experience severe myelosuppression following introduction of azathioprine or other thiopurines and this may help to tailor the dose or early switch to alternate therapy to achieve better treatment outcomes without life-threatening morbidity.

Ethical approval: Institutional Review Board approval is not required.
Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.
Financial support and sponsorship: Nil.
Conflicts of interest: There are no conflicts of interest.
Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Dean L, Kane M. Mercaptopurine therapy and TPMT and *NUDT15* genotype. Bethesda, MD: National Center for Biotechnology Information (US); 2012.
2. Devasia AJ, Illangeswaran RS, Raj IX, George B, Balasubramanian P. NUDT15 polymorphism explains serious toxicity to azathioprine in Indian patients with chronic immune thrombocytopenia and autoimmune hemolytic anemia: A case series. Drug Metab Pers Ther 2020;35:20200128.
3. Khaeso K, Udayachalerm S, Komvilaisak P, Chainansamit SO, Suwannaying K, Laoaroon N, *et al*. Meta-analysis of *NUDT15* genetic polymorphism on thiopurine-induced myelosuppression in Asian populations. Front Pharmacol 2021;12:784712.

4. Grover N, Bhatia P, Kumar A, Singh M, Lad D, Mandavdhare HS, *et al*. TPMT and NUDT15 polymorphisms in thiopurine induced leucopenia in inflammatory bowel disease: A prospective study from India. BMC Gastroenterol 2021;21:327.
5. Jena A, Jha DK, Kumar MP, Kasudhan KS, Kumar A, Sarwal D, *et al*. Prevalence of polymorphisms in thiopurine metabolism and association with adverse outcomes: A South Asian region-specific systematic review and meta-analysis. Expert Rev Clin Pharmacol 2021;14:491-501.
6. Pratt VM, Cavallari LH, Fulmer ML, Gaedigk A, Hachad H, Ji Y, *et al*. TPMT and *NUDT15* genotyping recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for pharmacogenomics and personalized therapy, and pharmacogenomics knowledgebase. J Mol Diagn 2022;24:1051-63.
7. Babu T, Durairaj L, Sanjeevi A, Subramanian J, Goswami U, George D, *et al*. A *NUDT15* genetic polymorphism study in adult acute leukemia patients integrating pharmacogenomics and clinical practice: A single centre experience from South India. Blood 2023;142:5799.

How to cite this article: Mishra N, Bansal N, Arya V, Kotwal J, Gupta N. Severe bone marrow aplasia following azathioprine therapy in patients with NUDT15 deficiency: A case series. J Hematol Allied Sci. 2025;5:77-80. doi: 10.25259/JHAS_51_2024