Case Report

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NUDT15 polymorphism explains serious toxicity to azathioprine in Indian patients with chronic immune thrombocytopenia and autoimmune hemolytic anemia: a case series

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Abstract

Objectives: Azathioprine (AZA) is a commonly used immunosuppressant in patients with autoimmune diseases. The toxic side effect to AZA (myelosuppression, hair loss, and oral ulcers) are highly unpredictable which can be life threatening if not identified earlier and dose adjustments made or the drug is withdrawn.

Case presentation: Here we report a case series of five patients with severe toxicity while on treatment with AZA for autoimmune hemolytic anemia (n=1) and Immune thrombocytopenia (n=4). The common thiopurine methyltransferase (TPMT) variants (TPMT*2, *3A, *3B) were not present in these patients. However, all these patients had the NUDT15 415C>T variant that has been reported to explain serious toxicity to thioguanine in Asian patients.

Conclusions: Our report suggests pre-emptive genotype-based dosing of AZA could reduce adverse toxicity and hence better outcome.

Keywords: azathioprine; NUDT15; polymorphism; thiopurine methyltransferase (TPMT).

Introduction

Azathioprine (AZA) is a common immunosuppressant belonging to a class of thiopurines used in the treatment of various autoimmune diseases like autoimmune hemolytic anemia (AIHA) and Immune thrombocytopenia (ITP). AZA is a prodrug that has to be first converted to form the active metabolite–thioguanine nucleotides (TGN). Thiopurine methyltransferase (TPMT) inactivates AZA resulting in less parent drug available to form 6-thioguanine nucleotide (TGNs) [1]. The most common toxicity encountered during AZA usage is dose dependent bone marrow suppression. This can usually be reversed by reducing the dose of AZA. While patients carrying two non-functional TPMT alleles can experience life-threatening myelosuppression due to high levels of TGNs, those patients carrying one non-functional TPMT allele may also be unable to tolerate conventional doses of AZA and may require dose reduction when treated with AZA [2]. However the common variants are extremely rare in the Asian population, including ours [3–5]. Polymorphism in multidrug resistance protein–4 (MRP4) and inosine triphosphate pyrophosphatase (ITPA) have also been shown to explain toxicity to AZA [6, 7]. Recently a polymorphism (c.451C>T) in nucleoside diphosphate-linked moiety X motif 15 (NUDT15) has been recognized to contribute to differences in thiopurine tolerance [8, 9] in patients of Asian ancestry. This variant frequency has been described commonly in East Asians and Hispanics, but rarer in the Europeans and could be the reason why Asians are highly intolerant to AZA compared to the Europeans despite having lower prevalence of TPMT mutant polymorphism. We describe, our experience with five adult patients, who were treated with AZA for various hematological disorders and developed myelosuppression requiring cessation/dose adjustments. All these patients were found to be negative for TPMT polymorphism, but found to have polymorphism in NUDT15.

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This is a retrospective case series of five patients (four patients with ITP and one with AIHA) who developed myelosuppression while on AZA therapy. AZA was started as the second line agent in these patients at a dose of 1–2 mg/kg with periodic monitoring of blood counts and subsequent doses adjusted if any patient develops symptoms of AZA toxicity (unexpected cytopenia, hair loss or oral ulcers). The study was approved by the Institutional Review Board.

Blood samples were collected from these patients in EDTA tubes when they presented with features of AZA intolerance. Genomic DNA was extracted and was used to screen for common TPMT variants as reported previously [3]. NUDT15 451C>T, MRP4 2269G>A and ITPA 94C>A variants were screened retrospectively in the available genomic DNA by bidirectional Sanger Sequencing as reported previously [6–8]. Clinical details were collected from patients' electronic medical records.

The demographics of the patients and the genotypes of TPMT, NUDT15, MRP4, and ITPA are listed in Table 1.

Case presentation

Case 1

A 26-year-old female presented to us in April 2017 with complaints of transfusion dependent anemia with jaundice. She was diagnosed as autoimmune hemolytic anemia and was started on corticosteroids (prednisolone at 1 mg/kg/day). She showed response to steroids, but while on steroid taper, her hemoglobin fell and she was started on second line therapy with AZA at 1.5 mg/kg/day. One week after initiating her on AZA, she presented to us with hair loss, oral ulcers and leukopenia (WBC-1900). AZA was stopped and her symptoms and white cells counts improved. She was further treated with steroids, Rituximab and mycophenolate mofetil following which her hemoglobin stabilized and she is in response.

Case 2

A 28-year-old primigravida presented to us in the second trimester of pregnancy with spontaneous abortion. Her platelet count was 13,000/cu.mm. She was diagnosed as Immune thrombocytopenia and she was started on oral corticosteroids (prednisolone 1 mg/kg/day). Her platelet counts improved to 85,000. However on steroid taper, her platelet counts dropped to <30000. She was started on AZA at 1.5 mg/kg/day. Two weeks later she presented to us with

Table 1: Summary of cases with NUDT15 mutations who developed Azathioprine (AZA) toxicity while on therapy.

No	Gender/ age, years	Diagnosis	AZA, mg/kg/day	Dose of AZA, mg/kg/day	Symptoms	Nadir WBC count	Nadir ANC	Median time from starting AZA to toxicity, days	TPMT*2, *3 genotypes	MRP4 2269G>A	ITPA 94C>A	NUDT 15 genotype	Intervention
1	F/26	AIHA	1.5	1.5	Hair loss	1900	190	7	WT	WT	WT	MUT	AZA stopped
2	F/28	Chronic ITP	1.5	1.5	Hair loss	1400	210	14	WT	WT	WT	MUT	AZA stopped
3	F/34	Chronic ITP	2	2	Fever	1900	190	14	WT	WT	WT	MUT	AZA stopped
4	F/18	Chronic ITP	1.5	1.5	Hair loss	5600	1680	21	WT	WT	HET	HET	AZA dose adjusted
5	F/12	Chronic ITP	2	2	Hair loss	2000	800	28	WT	WT	WT	HET	Lost to follow up

AIHA, autoimmune hemolytic anemia; ITP, immune thrombocytopenia; AZA, azathioprine; ANC, absolute neutrophil count; WT, wild type; TT, homozygous; CT, heterozygous.

hair loss and leukopenia (WBC 2400/cu.mm). AZA was stopped and her counts improved in a week. She was further treated with Dapsone with which she remains in partial response.

Case 3

A 34-year-old female presented to us in April 2016 with thrombocytopenia and bleeding manifestations. She was diagnosed as Immune thrombocytopenia and showed excellent response to corticosteroid therapy. However, she remained steroid dependent and was hence started on AZA at 2 mg/kg/day. She presented to us 2 weeks later with high grade fever and cytopenia (WBC – 1900/cu.mm, platelet count – 2000/cu.mm). AZA was stopped, and her white cell counts fully recovered. After stabilization she underwent a laparoscopic splenectomy following which she remains in complete response.

Case 4

An 18-year-old female was diagnosed as Immune thrombocytopenia in July 2015. She was treated with IV Immunoglobulins and corticosteroids with which she achieved complete response. The disease relapsed in August 2016 when she was restarted on corticosteroids along with AZA at 1.5 mg/kg/day. Three weeks later she presented with hair fall. Her blood counts were normal. AZA was continued at the reduced dose of 1 mg/kg/day which was tolerated without any cytopenia for the next 2 years. She achieved a complete response.

Case 5

A 12-year-old female was diagnosed as chronic Immune thrombocytopenia in September 2011. She has been symptomatic from 2006 and had been on multiple courses of corticosteroids at varying doses since then. She was initiated on AZA at 2 mg/kg/day. One month later she presented with significant hair fall and leukopenia (WBC – 2000). AZA was withheld and her blood counts improved in 2 days. She was further lost to follow up.

Discussion

AZA is a commonly used immunosuppressive agent in the treatment of various auto-immune diseases. The enzyme

TPMT plays a key role in AZA metabolism. Inherited genetic polymorphisms in the TPMT gene could cause abnormal metabolism and lead to accumulation of excess 6-TGN which in turn could increase the risk of AZA-induced adverse reactions, particularly leukopenia [10, 11]. Asians are found to tolerate AZA poorly and are more susceptible to toxicity, but paradoxically the common genetic polymorphisms in TPMT gene (TPMT *2, *3) are extremely rare in Asians compared to the frequency reported in the European population [12]. It has been reported recently that TGNs associated toxicity can be seen even in the absence of TPMT polymorphisms in the Asian population [8, 9]. We have previously reported that TPMT polymorphism alone cannot explain the variations in thiopurine related toxicity in a cohort of patients with acute lymphoblastic leukemia [3]. A Korean group had reported the association between thiopurine induced myelosuppression and a variant in the NUDT15 gene in patients who were on AZA for inflammatory bowel disorder [8]. The same genetic variant was also linked with 6-mercaptopurine (6-MP) related myelosuppression in patients of East Asian ancestry with acute lymphoblastic leukemia [13].

In this case series, the first three patients were homozygous for NUDT15 polymorphism and it can be seen that, cytopenia and other symptoms of toxicity occurred earlier (within 7–14 days) and it was more severe in them. AZA was stopped in all of them and was never rechallenged. One patient had only hair loss without cytopenia in the third week which required dose adjustment of AZA, whereas in the other patient, cytopenia developed in the fourth week and was non severe. Polymorphisms in NUDT15 can hence explain and predict the occurrence and severity of myelosuppression seen in Indian patients, in whom TPMT polymorphisms are rare compared to patients from the West.

Conclusions

This report indicates that NUDT15 polymorphism is an important determinant of myelosuppression related to the intake of thiopurines in patients with ITP and autoimmune hemolytic anemia. Preemptive genotyping for the common variants associated with 6-MP toxicity should help tailor the dose of thiopurines or early switch to alternative therapy to achieve better treatment outcomes without any dose-limiting side effects.

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Ethical approval: The study was approved by the Institutional Review Board.

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