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Research Article

Thiopurine S-Methyltransferase Genotype Mutation in Pediatric Patients with Inflammatory Bowel Disease on Azathioprine: A Pilot Study

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Abstract

Background: Inflammatory Bowel Disease (IBD) is a serious disease as well as the drugs used to treat it including azathioprine (AZA). Polymorphism of thiopurine S-methyl transferase enzyme (TPMT) gene has been reported to cause fatal complications in patients treated with AZA.

Aim of Work: The aim is to detect frequency and mutation of variants of TPMT and correlate the presence of side effects to the TPMT profile.

Materials and Methods: This cross sectional study included 19 IBD patients, with an age range of 6-17 years. They were 11 Crohn's disease patients and 8 ulcerative colitis patients. Complete blood count, alanine transferase (ALT), aspartate transferase (AST), ESR, CRP were done on initiation of AZA, at 2,4,16 weeks and at 7 months. Thiopurine methyl transferase (TPMT) genotyping was done (TPMT*2, TPMT*3A, TPMT*4A, TPMT*B and TPMT*3C).

Results: Variants of TPMT were wild type (100%). Myelosuppression was found in 10.5% of cases, hepatotoxicity in 21% of cases that proved on further investigations to be due to viral hepatitis.

Conclusion: Follow up of liver functions, CBC can be sufficient in IBD Egyptian children on AZA and can replace need for genotyping. Viral hepatitis is an important cause of elevated liver enzymes in IBD patients.

Keywords: AZA; TPMT; Inflammatory Bowel Disease

Introduction

Thiopurine drugs include immunosuppressant azathioprine (AZA), anticancer agents 6-mercaptopurine (6MP) and 6-thioguanine (6TG) which are currently widely used in the treatment of inflammatory bowel diseases (IBD) [1]. In the liver, AZA is initially metabolized to 6-MP through a non-enzymatic pathway, in which, 6-MP is converted to its metabolites via an actions of intracellular multi-enzymatic process [2]. thiopurine *S*-methyltransferase (TPMT) one of three enzymes play in this process, in which, the thiopurine dosage is ethintial to be administered based on an individual's TPMT activity level [3].

The principal and serious toxic effects of azathioprine are hematologic and gastrointestinal ones. Risk of secondary infection and malignancy is also significant. Frequency and severity of side effects depend on dose and duration of azathioprine as well as on underlying disease or concomitant therapies [4,5].

The FDA recommends TPMT genotyping or phenotyping before starting treatment with azathioprine). This allows patients who are at increased risk for toxicity to be identified and for the starting dose of azathioprine to be reduced, or for an alternative therapy to be used [2,6,7].

Four non-functional TPMT alleles have been identified (TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C). These alleles are believed to cause decreases in TPMT enzyme activity for between 80% and 95% [2]. The most common TPMT genotype in Caucasian populations is homozygous for the wild-type TPMT gene.

Where, Several studies have shown that the wild-type genotype have been found in 85-95% of patients, while In most populations, approximately 10% of individuals are heterozygotes and a further 0.3% carry homozygous variants of the non-functional TPMT alleles.

So in the current study it was hypothesized that, with the Egyptian oligo-ethnicity, IBD children would have a different mutational pattern of TPMT. The aim of this study was to detect the mutational pattern of TPMT and to monitor the clinical response to AZA and correlate the presence of side effects to the TPMT profile.

Materials and Methods

Subjects

This is a cross sectional study including 19 children with IBD, their ages ranged from 6 to 17 years. It was planned to start azathioprine for all of them, in a dose of 2-2.5 mg/kg, during the research duration (2017-2019). The study was carried out at the Pediatric Gastroenterology unit, Ain Shams University, Children's Hospital. Diagnosis of IBD was based on clinical, endoscopic, radiological and histological criteria [8].

The study was approved by the Ethics Committee of faculty of Medicine, Ain Shams University and an informed consent was taken from guardians of each participant in addition to asent from children above the age of 7 years.

Medical history taking as regards gastrointestinal symptoms associated with IBD (diarrhea, bleeding per rectum, abdominal pain, vomiting, hematemesis and abdominal distension), systemic manifestations related to IBD including fever, joint affection, weight loss, bony aches or fractures and symptoms associated with azathioprine toxicity (bone marrow suppression, pancreatitis and hepatotoxicity). Careful clinical examination for extra intestinal manifestations was done. Local abdominal examination was performed to detect distension, tenderness, rigidity and organomegaly.

Laboratory assessment to detect the azathioprine toxicity, complete blood count (CBC) and liver function test (LFT) monitored initially, 2, 4 and 16 weeks and at 7 months as well.

TPMT genotyping

Sample collection: 2 ml whole blood samples were collected from all patients using K2EDTA as anticoagulant inside vacutainer sterile tubes then stored at -20ºC for subsequent DNA extraction, genotyping detection of Thiopurine S-methyltransferase (TPMT) variants (*1 the wild type, *3A, *B,*2, *3C, *4A) Polymorphism chain reaction-restriction followed by DNA Sequencing was the used method.

Statistical analysis was carried out using the IBM SPSS (Statistical Package for the Social Sciences) Version. 25.0, (IBM Corp., USA, 2017-2018).

Results

The study included 11 patients with Crohn's disease (Group A) and 8 patients with ulcerative colitis (Group B). They were 9 males (47.4%) and 10 females (52.6%).

Among patients with Crohn's disease 27.3% (n = 3) were classified as early onset and 72.7% (n = 8) as paediatric onset disease, while in patients with ulcerative colitis the early onset disease was found in 62.5% (n = 5) and late onset was found in 37.5% (n = 3).

Results of thioprine s-methyl transferase variants *3A, *3C, *2, B, 4A showed wild type with no mutation detected in all patients.

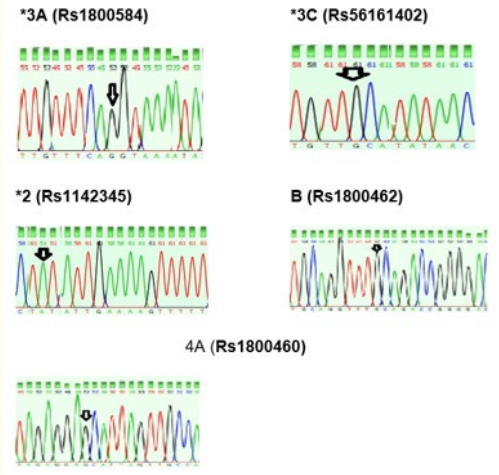

Figure 1: TPMT *3A, *3C, *2, B, 4A genotyping sequencing chromatography.

	0 month		7 months		TPMT variants (*3A,*B,*2,*3C,*4A)		Test value•	P value	Sig.
	No	%	No.	%	No.	allele			
Pancreatitis									
Yes	0	0.0%	0	0.0%	0		NA	NA	NA
No	19	100%	19	100%	19	*1/*1			
Hepatotoxicity									
Yes	0	0.0%	4	21.0%	4	*1/*1	4.470	0.034	S
No	19	100%	15	78.9%	15	*1/*1			
Myelosuppression									
Yes	1	5.3%	3	37.5%	3	*1/*1	1.117	0.290	NS
NO	18	94.7%	16	84.2%	16	*1/*1			

Table 1: The Side effects of azathioprine in relation to TPMT variants.

None of patients in the study showed TPMT variant mutation (*1 the wild type, *3A,*B,*2,*3C,*4A) 100% were of the wild-type homozygous TPMT*1/*1 genotype. At 7 months, hepatotoxicity evidence showed in 4 patients (21%) (one patient had HAV IgM +ve and 3 patients had HCV +ve PCR). Two more patients (10.5%) had myelosuppression (leukopenia) at 7 months, which was mild leukopenia (3.7 and 3.5 10^3/ul).

Initial laboratory testing for all patients showed that initial mean hemoglobin level was 9.94 gm/dl with severe anemia occuring in one patient. Mean total leukocytic count was 10.88 x 1000/cmm with leukopenia in 5.3%. Mean Platelet count was high (529.47 x 1000/cmm) with moderate thrombocytosis in 21%, severe thrombocytosis in 5.3% and thrombocytopenia in 5.3%).

Comparison between initial and follow up laboratory findings in Crohn's patients (Table 2) showed that 45.5% of patients turned to normal hemoglobin level, while one patient (9.1%) was still suffering from severe anemia after 7 months of follow up. The platelet count became normal in 72.7% of patients in comparison

to 18.2% before treatment. Leucopenia occurred in 18.2% of patients at 7 months. Two patients (18.2%) turned to be normal ESR level and to negative CRP at the end of follow up. All Crohn's disease patients showed normal liver functions tests initially and at 7 months follow up.

Crohn's disease No. = 11 No. = 11		**Initial**	**7 months**	**Test value**	**P value**	**Sig.**
Hemoglobin (gldl) Range	Mean ± SD	9.36 ± 1.87	10.49 ± 2.27	-1.401•	0.192	NS
	6 - 12.9	6.08 - 13.6				
Normal		2 (18.2%)	5 (45.5%)	2.571*	0.463	NS
Mild anemia		3 (27.3%)	3 (27.3%)			
Moderate anemia		5 (45.5%)	2 (18.2%)			
Severe anemia		1 (9.1%)	1 (9.1%)			
Total lekocytic count (10^3/ul).	Mean ± SD	11.65 ± 8.16	8.06 ± 2.84	1.319•	0.217	NS
	Range	5.1 - 27.8	4.8 - 13.8			
Normal		7 (63.6%)	7 (63.6%)	2.667	0.264	NS
Leucocytosis		4 (36.4%)	2 (18.2%)			
Leukopenia		0 (0.0%)	2 (18.2%)			
Platelet (10^3/ul).	Mean ± SD	629.82 ± 238.5	404.73 ± 161.3	2.863	0.017	S
	Range	198 - 973	260 - 797			
Normal		2 (18.2%)	8 (72.7%)	7.600	0.107	NS
Mild thrombocytosis		3 (27.3%)	2 (18.2%)			
Moderate thrombocytosis		4 (36.4%)	1 (9.1%)			
Severe thrombocytosis		1 (9.1%)	0 (0.0%)			
Thrombocytopenia		1 (9.1%)	0 (0.0%)			
PNL (103/ul).	Mean ± SD	3.90 ± 1.72	4.69 ± 2.74	-0.731	0.482	NS
	Range	0.4 - 6.3	1.5 - 11.3			
Normal		10 (90.9%)	10 (90.9%)	2.000	0.368	NS
Neutrophilia		0 (0.0%)	1 (9.1%)			
Neutropenia		1 (9.1%)	0 (0.0%)			
Absolute neutrophilic count (10^3/ul).	Median (IQR)	2.4 (1.7 - 3.6)	2.1 (1.5 - 3.2)	-1.201	0.230	NS
	Range	1.3 - 9.3	1.4 - 3.8			
Normal		10 (90.9%)	10 (90.9%)	0.000	1.000	NS
Lymphocytosis		0 (0.0%)	0 (0.0%)			
Lymphopenia		1 (9.1%)	1 (9.1%)			
ESR1st hour	Median (IQR)	50 (50 - 75)	30 (15 - 40)	-2.851	0.004	HS
	Range	25 - 85	5 - 60			
Normal		0 (0.0%)	2 (18.2%)	2.200	0.138	NS
High		11 (100.0%)	9 (81.8%)			

Table 2: Laboratory data initially and after 7 months in Crohn's disease patients.

Comparison between initial and follow up laboratory findings in ulcerative colitis patients as seen in table (2) showed that normal hemoglobin level was not changed in 50% of patients with ulcerative colitis at the end of follow up, one patient (12.5%) turned from moderate to mild anemia. The percentage of patients who had normal mean platelet count decreased from 62.5% to 37.5%, one patient (12.5%) turned to severe thrombocytosis and another one to moderate thrombocytosis (12.5%).

Neutrophilia appeared in one patient (12.5%). Neutropenia and lymphopenia were found in 37.5%, 25% respectively at 7 months of follow up. ESR was equally high in 87.5% initially and at 7 months. One patient (12.5%) had high level of liver function tests initially and at 7 months of follow up among patients with ulcerative colitis.

Ulcerative colitis (CBC) **No. = 8** **No. = 8**		**Initial**	**7 months**	**Test value**	**P value**	**Sig.**
Hemoglobin (g/dl)	Mean ±SD	10.74 ± 1.44	11.34 ± 1.23	-0.906•	0.395	NS
	Range	8.4 - 12.3	9.6 - 13.5			
Normal		4 (50.0%)	4 (50.0%)	2.667*	0.446	NS
Mild anemia		3 (25.0%)	4 (50.0%)			
Moderate anemia		1 (12.5%)	0 (0.0%)			
Severe anemia		0 (0.0%)	0 (0.0%)			
Total leukocytic count(10^3/ul)	Mean ±SD	9.83 ± 4.77	7.95 ± 3.10	1.673•	0.138	NS
	Range	4.7 - 20	4.2 - 12.5			
Normal		4 (50.0%)	5 (62.5%)	0.311*	0.856	NS
High		3 (37.5%)	2 (25.0%)			
Low		1 (12.5%)	1 (12.5%)			
Platelet (10^3/ul).	Mean ± SD	391.5 ±121.7	471.4 ±162.9	-1.372•	0.212	NS
	Range	247 - 578	202 - 670			
Normal		5 (62.5%)	3 (37.5%)	1.643*	0.440	NS
Mild thrombocytosis		3 (37.5%)	4 (50.0%)			
Moderate thrombocytosis		0 (0.0%)	0 (0.0%)			
Severe thrombocytosis		0 (0.0%)	0 (0.0%)			
Thrombocytopenia		0 (0.0%)	1 (12.5%)			
PNL(10^3/ul). Range	Mean ±SD	4.50 ± 1.81	3.15 ± 2.68	1.035•	0.335	NS
	2.3 - 8	0.7 - 8.4				
Normal		8 (100.0%)	4 (50.0%)	5.333*	0.069	NS
Neutrophilia		0 (0.0%)	1 (12.5%)			
Neutropenia		0 (0.0%)	3 (37.5%)			

Table 3: Laboratory follow up between initial and at 7 month in ulcerative colitis patients.

Laboratory findings initially and after 7 months of follow up concerning AZA side effects showed elevated liver transaminases in one patient (12.5%) with ulcerative colitis. Although pancreatic functions (serum amylase and lipase) were high in 1 patient (9.1%) with Crohn's, yet it didn't fulfil criteria to diagnose acute pancreatitis (3 folds the upper limits of normal as regard NASPGHAN guidelines 2018).

	Crohn's (Group A) No = 11	UC (Group B) No = 8	Test value*	P value	Sig.
Leukopenia (0M)	N = 0 (0.0%)	N = 1 (12.5%)	1.145	0.228	NS
Leukopenia (7M)	N = 2 (18.2%)	N = 1 (12.5%)	1.497	0.473	NS
Neutropenia (0M)	N = 1 (9.1%)	N = 0 (0.00%)	0.768	0.381	NS
Neutropenia (7M)	N = 0 (0.0%)	N = 3 (37.5%)	4.898	0.026	S
Lymphopenia (0M)	N = 1 (9.1%)	N = 0 (0.00%)	0.768	0.381	NS
Lymphopenia (7M)	N = 1 (9.1%)	N = 2 (25.0%)	0.882	0.348	NS

Table 4: Myelosuppression follow up in patient groups.

Leukopenia appeared in two patients (18.2%) with Crohn's disease at 7 months, was the same in one patient (12.5) with ulcerative colitis. Neutropenia was significantly higher in ulcerative colitis (37.5%) than in Crohn's disease (0.0%) at the end of follow up ($p < 0.05$). Lymphopenia occurred in 2 more patients (25%) with ulcerative colitis.

Discussion

The genetic variants in TMPT gene has been extensively studied, where, the Caucasian population show 4-11% a moderate TPMT activity, while approximately 0.3% have very low or absent TPMT activity. In the Asian population, a bone marrow toxicity caused by AZA has been observed significantly caused due to the TPMT polymorphism in about 1.5-3% of the individual. Thus, it has been recommended that the TPMT status of patients should be determined prior to the commencement of thiopurine therapy. Two meta-analyses reported that the incidence of TPMT polymorphism was not associated with AZA-induced hepatotoxicity in patients with autoimmune diseases and inflammatory bowel disease [1].

TPMT enzyme activity is related to 4A (rs1800462), B (rs1800460), and 3C (rs1142345) polymorphisms. AZA toxicity are determined by a large algorism of TPMT genetic heterogeneity, where, low TPMT activity is observed with homozygous patients with two mutant non-functional TPMT gene alleles, intermediate TPMT activity is observed with heterozygous individuals with one functional and one non-functional allele and normal or high TPMT activity is observed with homozygous wild-type (normal) individuals with two functional alleles [2].

In this study, TBMT variants (3A,4A,3C, B,*2) had been tested in all patients, where, all homozygous, no mutant alleles were detected in patients, myelosuppression (mild leukopenia) that didn't require interruption or modification of azathioprine therapy occurred in 2 patients (10.5%) and viral induced hepatotoxicity occurred in 4 patients (21%), pancreatitis was not observed in the studied patients.

This was in agreement with Tantawy [9] who designed to study frequency distribution of the most common variant alleles of

TPMT [TPMT*2, TPMT*3A, TPMT*3B and TPM*3C] in the Egyptian children with acute lymphoblastic leukemia who presented with clinical manifestation suggested myelosuppression toxicity, and required interruption or modification of azathioprine therapy. Among the 64 patients and the 70 controls, neither the studied leukemic patients nor the controls had the TPMT mutant variant alleles in either homozygous or heterozygous form.

Zalizko [2], found in their study that the homozygous wild-type TPMT*1/*1 genotype is the most frequently genotype in ulcerative colitis and Crohn's disease patients' groups, and that TPMT*3A is the most pervasive polymorphism in the study population.

In contrary to El-Rashedy [10] study conducted among Egyptian pediatric population on AZA found that twenty (80%) of the included 25 patients had a polymorphic TPMT allele. TPMT*3A was the most frequent (14/25, 56%), 8 patients were homozygous and 6 were heterozygous. TPMT*3C mutant allele was found in 4 patients (16%) in the heterozygous state while 2 patients (8%) were found to be heterozygous for TPMT*3B mutant allele. TPMT mutant patients, especially homozygous, were at greater risk of 6-Mercaptopurine hematological toxicity without significant difference regarding hepatic toxicity. Also, Sheu [1] said that, in their study an association induced hepatotoxicity caused by TPMT polymorphisms and thiopurine.

Vögelin [11] reported that 43% of patients taking AZA developed lymphopenia. Also, Rifai [12] concluded that AZA related Lymphopenia is a recognized effect of this treatment, but lymphopenia-related complications in IBD patients have not been widely reported and lymphopenia lasted on average of 85.4 days and spontaneously resolved in 13 patients.

Meggitt [13] concluded that neutropenia and lymphopenia were relatively common with AZA, In pediatric studies rates of lymphopenia vary from 1% to 43%, and neutropenia 5%, a recent systematic review by Schram [14] recorded an abnormal CBC in 77% of patients on AZA therapy.

Rifai [12], a study of 52 adults with inflammatory bowel disease, lymphopenia was significantly associated with concurrent steroid treatment at the start of azathioprine use. As patients were on systemic steroid treatment for the initial weeks of azathioprine therapy, this could contribute to the rate of mild lymphopenia.

Conclusion

Close follow up for occurrence of side effects of treatment with AZA is mandatory regardless their TPMT mutational pattern. Further studies are needed in Egypt to detect the true prevalence of mutant variants of TPMT among Egyptians.

Bibliography

1. Singh A., *et al*. "Use of thiopurines in inflammatory bowel disease: an update". *Intestinal Research* 20.1 (2022): 11-30.

2. Zalizko P., *et al*. "Thiopurine S-methyltransferase genetic polymorphisms in adult patients with inflammatory bowel diseases in the Latvian population". *Therapeutic Advances in Gastroenterology* 14 (2020): 13.

3. Sheu HS., *et al*. "Thiopurine S-Methyltransferase Polymorphisms Predict Hepatotoxicity in Azathioprine-Treated Patients with Autoimmune Diseases". *Journal of Personalized Medicine* 12.9 (2022): 1399.

4. Kolbeinsson HM., *et al*. "Pancreatic Cancer: A Review of Current Treatment and Novel Therapies". *Journal of Investigative Surgery* 36.1 (2023).

5. Dayharsh GA., *et al*. "Epstein-Barr virus-positive lymphoma in patients with inflammatory bowel disease treated with azathioprine or 6-mercaptopurine". *Gastroenterology* 122.1 (2002): 72-77.

6. Bruijstens AL., *et al*. "E.U. paediatric MOG consortium consensus: Part 5 - Treatment of paediatric myelin oligodendrocyte glycoprotein antibody-associated disorders". E*uropean Journal of Paediatric Neurology* 29 (2020): 41-53.

7. Dean L. "Azathioprine Therapy and TPMT Genotype". *Medical Genetics Summaries* (2016).

8. Jevon GP and Madhur R. "Endoscopic and Histologic Findings in Pediatric Inflammatory Bowel Disease". *Gastroenterology and Hepatology* 6.3 (2010): 174-180.

9. Tantawy AA., *et al*. "Influence of thiopurine methyltransferase gene polymorphism on Egyptian children with acute lymphoblastic leukaemia". *Journal of Genetics* 96.6 (2017): 905-910.

10. El-Rashedy FH., *et al*. "Clinical implication of thiopurine methyltransferase polymorphism in children with acute lymphoblastic leukemia: A preliminary Egyptian study". *Indian Journal of Medical and Paediatric Oncology* 36.4 (2015): 265-270.

11. Vögelin M., *et al*. "The Impact of Azathioprine-Associated Lymphopenia on the Onset of Opportunistic Infections in Patients with Inflammatory Bowel Disease". *PLoS One* 11.5 (2016): e0155218.

12. Rifai A., *et al*. "Natural history of azathioprine-associated lymphopenia in inflammatory bowel disease patients: a prospective observational study". *European Journal of Gastroenterology and Hepatology* 23.2 (2011): 153-158.

13. Meggitt SJ., *et al*. "Azathioprine dosed by thiopurine methyltransferase activity for moderate-to-severe atopic eczema: a double-blind, randomized controlled trial". *Lancet* 367.9513 (2006): 839-846.

14. Schram ME., *et al*. "Off-label use of azathioprine in dermatology: a systematic review". *Archives of Dermatology* 147.4 (2011): 474-488.