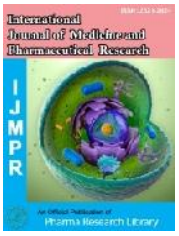
International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr



Research Article **Open Access**

Thiopurine methyl transferase enzyme activity-relationship to the dose and adverse effects of 6-mercaptopurine in children with acute lymphoblastic leukemia

Osama A.E. Ali*, Nabeel A.J.Ali[1], Janan G. Hassan[2]

[1]MBChB, PhD, Department of Pharmacology, College of Medicine, University of Basrah, Basrah, Iraq
[2]MBChB,FICMS, Department of Pediatrics, College of Medicine, University of Basrah, Basrah, Iraq

A B S T R A C T

Background: There are genetic variations in the activity of the enzyme thiopurine methyl transferase (TPMT) which lead to differences in the rate of metabolism of the thiopurine drugs, especially Mercaptopurine (6-MP) which may predispose patients to the toxic effects of these drugs or therapeutic failure might occur in the children with acute lymphoblastic leukemia (ALL). **Methods:** Children with ALL (80) were included in the study ,of them (15)patients were newly diagnosed and (65)patients on maintenance treatment ,we also studied apparently normal (78)children .Serum samples were collected from each patient and healthy children for the estimation of TPMT activity by ELISA (Enzyme –linked immune sorbent assay) and measurement various blood parameters **Results:** In normal children group, TPMT concentration was distributed as 28.3% had low, 17.9% had intermediate and 53.8% had normal or high activity. In the newly diagnosed group have TPMT concentration distributed as 66.7% low, 13.3% intermediate and 20% had normal or high activity. They have a reduction in all blood parameters, for Hb level (9.92± 2.06 g/dL) ,WBC count (4184 ± 1068.6 /mm³), Neutrophil (1537.3 ± 2142.7/mm³) and platelets(157533 ± 99609/mm³).The maintenance group have TPMT concentration distributed as 67.7% low, 21.5% intermediate and 10.8% had normal or high activity , they had a reduction in all blood parameters, for Hb level (10.49 ±1.73g/dL),WBC count (3657 ± 1036.8/mm³), Neutrophil (1434.7 ± 1180.7/mm³) and for platelets (219292 ± 133981/mm³).There was a significant ($p < 0.001$) correlation between TPMT concentration level and blood parameters (Hb , WBC, neutrophil and platelets) in the maintenance group of ALL children. **Conclusions:** Despite the variation in the level of TPMT in patients with ALL, it is useful as guide for the dose of 6-mp determination initially in the newly diagnosed and for the dose adjustment in the maintenance group.

Keywords: TPMT, ALL, 6-MP, Children, Leukemia

A R T I C L E I N F O

CONTENTS

Article History: Received 28 December 2016, **Accepted** 29 January 2017, **Available Online** 10 February 2017

***Corresponding Author**
Mr. Osama A.E. Ali
BSc (Pharmacy),
Basrah Health Directorate Basrah, Iraq
Manuscript ID: IJMPR3298

PAPER-QR CODE

Citation: Osama A.E. Ali, *et al.* Thiopurine methyl transferase enzyme activity-relationship to the dose and adverse effects of 6-mercaptopurine in children with acute lymphoblastic leukemia. *Int. J. Med. Pharm. Res*., 2017, 5(1): 59-64.

1. Introduction

Acute lymphoblastic leukemia(ALL) it is a wide spread cancer in children with more than 6000 new ALL diagnosed case each year in United States and regarded as the first cause for children death from cancer.[1]ALL can be diagnosed by CBC (complete blood count) and bone marrow tests (aspiration and biopsy).[2]Patients with ALL need to start treatment as soon as possible after diagnosis.[3]The maintenance Therapy consists from daily dose 6-mercaptopurine (6-MP) tablet.[4]Patients with ALL in the remission stage need to be followed-up in hospital for regular checking. Bone marrow aspiration may be needed from time to time during treatment[5]. 6-MP is an anti-cancer, antineoplastic, cytotoxic chemotherapy drug. It is classified as an antimetabolite with immunosuppressant features. It is commonly used along with other medicines in the management of acute lymphoblastic leukemia. Thiopurine methyl transferase (TPMT) is cytoplasmic enzymes which stimulate the S-methylation of aromatic and heterocyclic sulfhydryl compounds.[6] TPMT is part of the main enzymes involved in the metabolism of thiopurine drugs including azathioprine (AZA) and 6-Mercaptopurine (6-MP).[7] TPMT is explicated in several cells; where in the liver TPMT levelly is high while the brain and lung are low.[8] TPMT made up of 10 exons in the size of 25 kb. [3][9] Severe myelosuppression is a dose-dependent reaction which may be caused by the active metabolite, deoxy-6-thioguanosine 5' triphosphate (6-TGN).

Overdosing might give rise to excess levels of 6-TGN, which also lowered the inactivation of the drug. These drugs required conversion to TGN to maintain their remedial effects, therefore the function of TPMT is to inactivate thiopurine drugs that include azathioprine (AZA) and 6-mercaptopurine (6-MP), by methylation. [10]On the basis of TPMT enzyme activity, patients can be classified as 3 distinct phenotypes: 1st group had normal/ high activity (with 2 functional alleles of the active gene) occur at 89%, 2nd group had intermediate activity (heterozygous, with 1variant allele) occurs at 11% and 3rd group had no/low activity (homozygous, with 2 variant alleles) occur at 0.3% in the population. Factors that effect on TPMT activity include Blood transfusion[11], Liver disease: such as hepatitis can effect on the patients having to screen for TPMT status because of the TPMT enzyme activity as the liver as rich in TPMT enzyme and Drugs interaction.[12,13] The aim of this study to explore the clinical importance of TPMT measurement in guiding the treatment of children with ALL and whether pretreatment determination of (TPMT) enzymatic activity (phenotyping) can guide the dose 6-MP also To evaluate the changes of the enzyme activity during maintenance treatment with 6-MP and to predict the effect of 6-MP on various blood parameters.

2. Materials and Methods

Methods: Pediatric cancer patients aged from (0.5-15) years old was admitted to Basra Specialty Hospital for the children for chemotherapy. Children with acute lymphoblastic leukemia treated with 6-Mercaptopurine. The patients were divided into three groups according to the duration of chemotherapy, group 1 patients at time of diagnosis and before the start of any treatment, group 2 patients at the maintenance therapy usually after start of treatment for at least one month And g group 3 Normal healthy children. Control group samples of blood were collected from children attending hospital for either routine medical checkup or accompanying their brothers or sisters and also some sample were collected from primary school children. Exclusion criteria include children with hepatitis B or C, recent history of blood transfusion (within one month) and drugs that influence TPMT activity. Blood collection: 2 ml of blood was accumulated from each child via intravenous puncture. The blood was transfer to gel tube and left for 20 minutes to clot at room temperature. Then, the sample put in centrifuge (at 2000 RPM) for 15 minutes to separate the serum. The serum was carefully collected into plain tube and the serum was reserved at -20 C in refrigerator until assayed. Parameters that measured in the study include TMPT concentration by ELISA method, Hemoglobin, WBC count. Platelets count and Neutrophil.
Results are achieved by interposition from the standardization curve as show in figure (1) Analysis was carried out by using biostatistics programmer SPSS computer package version 15.

3. Results and Discussion

RESULTS

A total of 158 children (99 males and 59 females) were enrolled in this study. Their ages were from 6 months to 15 years, and the cases designed to 3 groups included a newly diagnosed group (N) ,15 children wasdiagnosed and confirmed by bone marrow aspiration as new cases of acute lymphoblastic leukemia (ALL) before taking any cytotoxic drugs. Maintenance group (M), 65 patients with ALL who are maintained on cytotoxic drugs and control group (C), and 78apparently healthy children. These treatments were given according to the treatment protocols used by Oncology department at Basra Specialty Hospital for Children; the different general characteristics of children were shown in (Table 1). This study divided the TPMT activity level to four categories according to reference value for TPMT, Low level of TPMT This category range from 1ng/ml to 30ng /ml for TPMT activity, intermediate level of TPMT This category range from 31ng/ml to 60ng/ml for TPMT activity, normal level of TPMT This category range from 61ng/ml to 90ng/ml for TPMT activity and high level of TPMT This category

range from 91ng/ml to 120ng/ml for TPMT activity. The distribution of TPMT activity between different groups of patients shown in as shown in (Table 2). There is 44.1 % of children that included in this study having a low TPMT activity,19 % of children having an intermediate TPMT activity , 11.2 % of children having a normal TPMT activity and finally 21.5 % of children having a high TPMT activity as in (Table 3). This study had obtained the following statistically significant results, hemoglobin level was reduced as TPMT activity decreased in the maintenance group; the WBC count was reduced as TPMT activity decreased in the maintenance group, the Neutrophil count was reduced as TPMT activity decreased in the maintenance group and the Platelets count was reduced as TPMT activity decreased in the maintenance group, the relationship between TPMT activity and other blood parameters in the maintenance group as in (Table 4). There are strong correlation between TPMT activity level and WBC count and platelets as shown in (Fig 1) and (Fig 2).

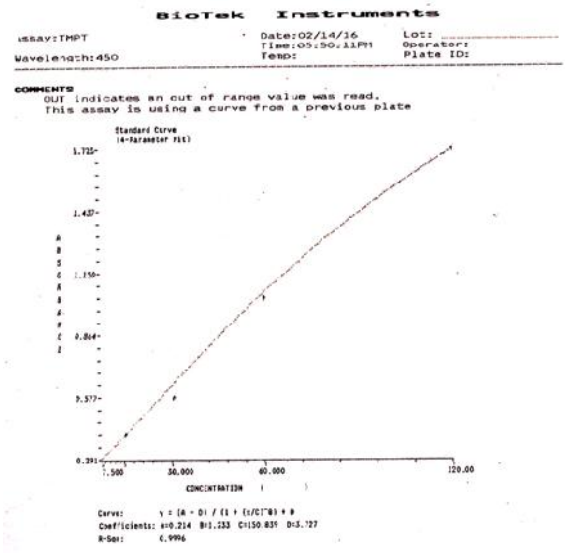


Figure 1: Standard calibrator curve for Thiopurine methyl transferase concentration estimation.

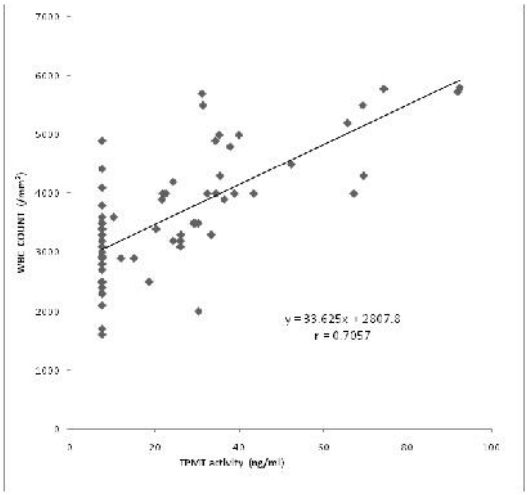


Figure 2: Correlation between TMPT activity level and WBC count in the maintenance group P<0.001 (significant) n=65

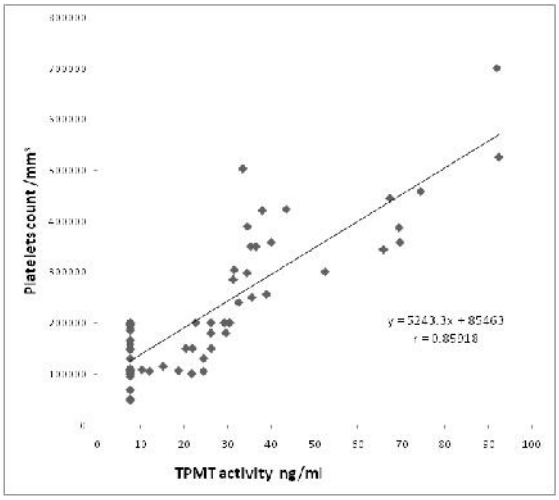


Figure 3: Correlation between TMPT activity level and platelets count in the maintenance group P<0.001 (significant) n=65

Discussion

In the present study we included children with ALL as they treated with various cytotoxic drugs during maintenance therapy, including 6mp, which has toxicity against various blood parameters result from myelosuppression.[14] There are different factors that can influence the metabolism of 6mp in the body, the most important of which is the TPM enzymatic activity.[15] Therefore we tried to answer the questions: Does TMPT activity influence the metabolism or toxicity of 6mp? Can we use TPMT activity assay as a routine test in patients with ALL to improve therapy and reduce toxicity. The distribution of TPMT activity among the Caucasian normal children was found as about 89% had high or normal activity of the enzyme and about 10% had intermediate activity and less than 1% had low or deficient activity.[16] However the TPMT activity in our study showed 54% having high or normal activity, and 18% intermediate and 28% low or defiant enzyme activity. These differences may be due to the small number of children included in this study (78 only), or it may be due to a real genetic variations.[17] The TMPT activity was differently distributed in the newly diagnosed children with ALL, which include 20% had high or normal, and 13% had intermediate and 67% had low or deficient activity of the enzyme. This may be explained on the basis of the disease effects which can distort the normal blood picture completely.[18]There are various disease status that also may influence TPMT enzyme activity as multiple sclerosis.[19] Patients with TPMT deficiency have elevated concentrations of TGNs and have severe toxicity when treated with the usual dosages of Mercaptopurine while high TPMT activity was associated with lower TGNs concentrations and have a higher risk of treatment failure, therefore the TPMT value can be predictive of life threatening bone marrow toxicity due to 6-MP .[20]Leukopenia and hepato toxicity were among the serious side effects of the 6-MP .[21] It has not been possible to predict individual differences either in the patients response to 6-MP or its adverse effect, one important factor which determine the differences in response to thiopurine might be individual genetic variations of TPMT

polymorphism.[22] Patients who had (one or two variant alleles) are classified as unstable and/or nonfunctional TPMT proteins, and are increasing risk of blood toxicity, and therefore require a reduction of the Mercaptopurine dose.[23]Children with TPMT deficiency showed myelosuppression due to elevated in 6-TG levels even at a much reduced 6-MP dose.[24] The relationship between TPMT concentration and the active metabolite is inverse, for the maintenance group following the starting of treatment course the distribution of enzymatic activity among patients was different from both the normal group and the newly diagnosed patients.[25] The distributions include about 11% had normal or high TPMT activity, 21% with intermediate activity and 68% had low activity. This is mainly attributed to the drug effect as it is known that prolong treatment with 6mp will suppress various blood parameters in addition to probably consume the excess enzyme and lead to reduction of its activity.[26]There were significant direct negative correlation between TPMT value and different blood parameters including, Hb, WBC, neutrophils and platelets. As the TPMT value decrease the blood parameter reduced.[26] Patients with lower TPMT activity have low blood parameters and probably more exposed to the toxicity of 6mp as compared to the normal or high activity. Therefore estimation of The TPMT activity is useful before the starting the treatment with chemotherapy and during follow-up of treatment.[27]There are many protocols that are used for the treatment of patients with ALL, including the UKALL2003 protocol, which is used in the Basra Specialty Hospital for Children. The treatment should be well adjusted to avoid the drug toxicity or treatment failure.

In conclusion, there is a direct relationship between serum level of TPMT and Hb level, WBC count, neutrophil count and platelets in children as newly diagnosed with acute lymphoplastic leukemia and after that during maintenance therapy and the measurement of TPMT activity is useful before starting treatment with 6-MP in children with ALL and it is also useful in follow up of these patients.

Table 1: Characteristics of children included in the study

	Group	Gender				Age (year)	Weight (kg)	Height(cm)
		Male		Female		Mean ±SD	Mean ± SD	Mean ± SD
		NO.	%	NO.	%			
1	Newly diagnosed group	9	60%	6	40%	7.4 ± 4.0	23.9 ± 13.4	112.7 ±,27.5
2	Maintenance group	36	55%	29	45%	6.8 ± 3.4	21.6 ± 12.0	112.2 ± 19.7
3	Control group	54	69%	24	31%	7.6 ± 2.8	28.8 ± 9.2	122.3 ± 15.1
	total	99		59		-	-	-

Table 2: Distribution of TPMT activity among different patients groups

	TPMT activity (ng/ml)	Newly diagnosed group		Maintenance group		Control group		Total children	
		No.		No.		No.	%	No.	%
1	1-30	10	66.7%	44	67.7%	22	28.3%	76	44.1%
2	31-60	2	13.3%	14	21.5%	14	17.9%	30	19.0%
3	61-90	2	13.3%	5	7.7%	11	14.1%	18	11.4%
4	91-120	1	6.7%	2	3.1%	31	39.7%	34	21.5%
	total	15		65		78		158	

Table 3: The relationship between TPMT activity in the newly diagnosed group and a various blood parameters

	TPMT activity (ng/ml)	N0.	Hb (g/dl)	WBC (/mm^3)	Neutrophil (/mm^3)	Platelets (/mm^3)
			Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
1	1-30	10	9.8 ± 6.9	3777 ± 781.7	755 ± 266	141400 ± 80807
2	31-60	2	8.3 ± 3.8	4250 ± 353.5	850 ± 71	79000 ± 59397
3	61-90	2	10.5 ± 1.3	4750 ± 353.5	3590 ±3804	195500 ± 707
4	91-120	1	13	7000	7240	400000
	total	15	9.9± 2.0	4184 ± 1067	1537 ± 2143	157533 ± 99609

Table 4: The relationship between TPMT activity in the Maintenance group and a various blood parameters

	TPMT activity (ng/ml)	NO	Hb (g/dl)	WBC (/mm^3)	Neutrophil (/mm^3)	Platelets (/mm^3)
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	1-30	44	9.8 ± 1	3146 ± 709	823.6± 193.2	141136± 45692
2	31-60	14	11.7 ± 0.6	4492 ± 681	19020.2 ± 766.7	344786 ± 75684
3	61-90	5	12.8 ± 0.7	4956 ± 771	3158 ± 706.3	398400 ± 51100
4	91-120	2	13.6 ± 1	5765 ± 50	6150 ± 353.6	612500 ± 123744

4. Acknowledgements

All thanks to all members of Laboratory Unit in Basrah Specialty Hospital for Children for their cooperation and help.

5. Declarations

Funding: University of Basrah /Iraq
Conflict of interest: None declared
Ethical approval: Ethical committee of University of Basrah, College of Medicine

6. References

[1] Gabert, J, E Beillard, VHJ Van der Velden, W Bi, et al. Standardization and quality control studies of 'real-time'quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia–a Europe against Cancer program. Leukemia 2003;17: 2318-57

[2] Dobson, Stephanie M, Robert Vanner, Esmé Waanders, et al. Abstract A25: Evolving functional heterogeneity in B-acute lymphoblastic leukemia. Cancer Research. 2016, 6: 24-25.

[3] Attarbaschi, Andishe, Georg Mann, Michael Dworzak, Peter Wiesbauer, Martin Schrappe, and Helmut Gadner. Mediastinal mass in childhood T-cell acute lymphoblastic leukemia: Significance and therapy response. Med PediatrOncol2002;39: 558-65.

[4] Esbenshade, Adam J, Debra L Friedman, Webb A Smith, et al. Feasibility and initial effectiveness of home exercise during maintenance therapy for childhood acute lymphoblastic leukemia. Pediatr Phys Ther 2014;26: 301.

[5] Davila, Marco L, Isabelle Riviere, Xiuyan Wang, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sc Transl Med, 6: 224-25.

[6] Liu, Chengcheng, Deqing Pei, Cheng Cheng, et al.Tolerability of 6-Mercaptopurine (6MP) in Patients with Thiopurine Methyltransferase (TPMT) Heterozygosity in the Context of Multi-Agent Therapy for Acute Lymphoblastic Leukemia (ALL). Blood 2014; 124: 3722-22.

[7] Ford, LT, and JD Berg. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment; a pharmacogenomic test whose time has come. J ClinPathol 2010; 63: 288-95.

[8] Petit, Elise, Sophie Langouet, HananeAkhdar, et al. Differential toxic effects of azathioprine, 6-mercaptopurine and 6-thioguanine on human hepatocytes.ToxicolIn Vitro2008; 22:632-42.

[9] Krynetski, Eugene, and William E Evans. Drug methylation in cancer therapy: lessons from the TPMT polymorphism. Oncogene 2003; 22: 7403-13

[10] Farfan, Mauricio J, Carolina Salas, Cristina Canales, et al. Prevalence of TPMT and ITPA gene polymorphisms and effect on mercaptopurine dosage in Chilean children with acute lymphoblastic leukemia. BMC Cancer 2014; 14: 1.

[11] Ferroni, MA, G Marchi, E Sansone, et al.Variability in the rate of 6-mercaptopurine methylation in the erythrocytes, liver and kidney in an Italian population. Eur J Clin Pharmacol 1996; 51: 23-29.

[12] Jannone, G, K Kafka, and KB Schwarz. Utility of Monitoring Azathioprine Metabolites in the Management of Children with Autoimmune Hepatitis. J HepatolGastroint Dis 2016; 2: 2.

[13] Barlow, NL, V Graham, and JD Berg. Expressing thiopurine S-methyltransferase activity as units per litre of whole-blood overcomes misleading high results in patients with anaemia. Ann ClinBiochem 2010; 47: 408-14.

[14] McLeod HL, Miller DR, Evans WE. Azathioprine-induced myelosuppression in thiopurine methyl transferase de- ficient heart transplant recipient. Lancet 1993; 341:1151.

[15] Dubinsky, M.C., Yang, H., Hassard, P.V., et al. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. Gastroenterology 2002; 122: 904-15.

[16] Schaeffeler, Elke, Christine Fischer, Dierk Brockmeier, Comprehensive analysis of thiopurine S-methyl transferase phenotype–genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenet Genomics. 2004;14: 407-17.

[17] Relling, Mary V, Michael L Hancock, James M Boyett, et al. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood1999; 93: 2817-23.

[18] Smith, Malcolm, Diane Arthur, Bruce Camitta, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin. Oncol 1996;14: 18-24.

[19] Menor, Larry J, Mohan V Tatikonda, and Scott E Sampson. New service development: areas for exploitation and exploration. J OperManag 2002; 20: 135-57.

[20] Richard, Vijay Samuel, Deana Al-Ismail, and Ahmed Salamat. Should we test TPMT enzyme levels before starting azathioprine?. Hematology 2007;12: 359-60.

[21] Mazor, Yoav, Eduard Koifman, Hela Elkin, et al. Risk factors for serious adverse effects of thiopurines in patients with Crohn's disease. Current drug safety. 2007, 8: 181-85.

[22] Corominas, Hèctor, Montserrat Domènech, Dolors González, et al. Allelic variants of the thiopurine s-methyl tranferase deficiency in patients with ulcerative colitis and in healthy controls. Am J Gastroenterol2002;95: 2313-17.

[23] Evans, WE. Treatment of acute lymphoblastic leukemia. N Engl J Med2006;354: 166-78.

[24] Cuffari C., Theoret Y, Latour S., Seidman G. 6-Mercaptopurine metabolism in Crohn's disease: correlation with efficacy and toxicity. Gut 1996;39: 401–406

[25] Levinsen, Mette, Mervi Taskinen, Jonas Abrahamsson, et al. Clinical features and early treatment response of central nervous system involvement in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2014; 61: 1416-21.

[26] Hawwa, Ahmed F, Jeff S Millership, Paul S Collier, Pharmacogenomic studies of the anticancer and immunosuppressive thiopurinesmercaptopurine and azathioprine. Br J Clin. Pharmacol 2008; 66: 517-28.

[27] Fakhoury, M, J Andreu-Gallien, A Mahr, et al. Should TPMT genotype and activity be used to monitor 6-mercaptopurine treatment in children with acute lymphoblastic leukaemia. J Clin Pharm Ther 2007;32: 633-39