Thiopurine S-Methyltransferase Genotyping in Iraqi Childhood Acute Lymphoblastic Leukemia Patients ; A Single Institute Study

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common cancer seen in children worldwide and is the most common cancer in children under 14 years of age. Although there have been major advances in treatment approaches for childhood ALL, serious toxicities such as profound leukopenia frequently affect treatment and lead to life threatening consequences such as severe infections and even death. There has been a lot of interest in inter-individual differences in drug metabolizing enzymes in order to better adjust drug dosage and therapy. In this regards, Thiopurine S-Methyltransferase *(TMPT)* was the first pharmacogene that showed a substantial association with Mercaptopurine (6-MP) maximum tolerated dose and 6-MP related toxicities leading to the implementation of *TPMT* genotyping before drug administration **Aim**: To identify the most common TPMT polymorphism (TPMT*3A, TPMT*3B and TPMT*3C) and its frequencies in a sample of Iraqi ALL paediatric patients. **Methods**: A cross sectional study was performed for 79 patients with Acute lymphoblastic leukemia . Genotyping for (*3A , *3B , *3C) the TPMT gene was performed by the allele-specific multiplex-PCR analysis method. **Results:** The TPMT*3A mutant allele was found in 18 patients with allele frequency of (22.8 %), while TPMT*3C mutant allele was detected in 5 children with allele frequency of 6.3% . But TPMT*3B mutant allele was not detected in whole sample ALL patients .The correlation between gender and the polymorphism was not statically significant as p-value 0.23.. **Conclusion:** TMPT genotyping is an essential tool to reduce the cytotoxic effects of the anti-cancer drug 6-MP in Iraqi paediatric patients with ALL .

Keywords: *6-Mercaptopurine **,** Acute lymphoblastic leukemia**,** neutropenia ,drug -toxicity*

Introduction

Acute lymphoblastic leukemia (ALL) ,is a malignant disease in bone marrow where the early lymphoid predecessors proliferate and exchange the normal hematopoietic cells of the marrow. It's cancer of blood cells owing to an increase in the number of white blood cells, they are gathering out the red blood cells and platelets that is usually needed for the healthy body, and all those additional white blood cells cause problems . ALL is the most common type of cancer, e.g. in the United States about 2,400 children and adolescents diagnosed with ALL every year[1]. In a child with this type of leukemia, leukemia cells split up to make copies of themselves. These copies split over, again and again, producing more and more cells . Not like normal blood cells, leukemia cells don't die as they become old or damaged [2]. The 6-Mercaptopurine (6-MP) - is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug[2]. It is also known as 6-Methylmercaptopurine

or Thiopurine S-methyl .The 6-MP molecular formula is C5H4N4S and molecular weight of 152.175 g/mol [3]. Thiopurine-S-methyltransferase (TPMT), is an enzyme that inactivates the drug. For instance, testing for specific decreased enzyme function polymorphisms prior to therapy, mainly *TPMT*2*, **3A*, **3B,* and **3C* has been included in several clinical guidelines and drug labels (PharmGKB, 2016; PharmGKB, 2018). (TPMT) catalyzes thiopurine and thiopyrimidine *S*-methylation, an important metabolic pathway for thiopurine drugs, such as *6*-t*hioguanine* (6-TG), and azathioprine, currently used to treat childhood acute lymphoblastic leukemia, .[4]

Materials and Methods

A cross sectional study in Children Welfare Teaching Hospital, Medical City, Baghdad, including pediatric patients, aged less than 14, being treated for Acute Lymphoblastic Leukemia during Induction, consolidation and delayed intensification phases and before starting the continuation (maintenance) phase of treatment which involve the use of 6-MP drug as the backbone for a duration of 1-2 years. The study include 79 subjects was conducted to study the association of (**3A*, **3B,* and **3C*) SNP of TPMT with Acute lymphoblastic leukemia. The patient population included 79 subjects(30 girl and 49 boys) with Acute lymphoblastic leukemia. Inclusion criteria constituted all patients with Acute lymphoblastic leukemia on chemotherapy (induction and consolidation phases) before starting maintenance phase of treatment, in which the 6-MP represent the back bone of treatment. Exclusion criteria represent any patient whose treatment was postponed due to Jaundice and / or impaired liver function due to hepatitis infection . Additionally, any patient who was recently received blood (within the last fourweeks) was excluded from the study

Genotypic data

Peripheral blood samples of ALL were collected in EDTA-anticoagulant tube and DNA was extracted from whole-blood samples using the Reliaprep genomic DNA extraction Kit (Promega, U.S.A). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U. K.). Genotyping was performed using allele-specific multiplex-PCR analysis method. For TPMT gene using thermocycler (Biometra, Germany) was in use . The primer sequences were obtained from [5]: TPMT*1 F- 5′GTATGA TTT TAT GCA GGT TTG 3′ and R- 5′TAA ATA GGA ACC ATC GGA CAC 3 TPMT*3B F-5`-GGGACGCTGCTCATCTTCT-3` and

R-5`-GCCTTACACCCAGGTCTCTG-3` TPMT*3C F- 5`-AAGTGTTGGGATTACAGGTG-3`and R- 5`-TCCTCAAAAACATGTCAGTGTG-3` Amplification was performed in a total volume of 23 µlwhich contained 12.5 µl of Go Taq Green Master Mix, (Promega Corporation, Madison, WI), 1µl of each primer (One Alpha, U.S.A.),3.5 µl of nuclease free water and 5 µl of DNA template. PCR amplification consisted of initial-denaturation step at 95°C for five minutes followed by 35 cycles of denaturation at 95°C for one minute, annealing at the specified annealing temperature for two minutes, followed by extension at 72°C for one minute and the final extension step at 72°C for five minutes. The PCR amplicons were separated on 2% agarose gel through electrophoresis. The bands were visualized with an Ultraviolet (UV) transilluminator at 365 nm (Cleaver Scientific, UK). The TPMT*1 allele was analyzed by allele-specific PCR with an annealing temperature of 53°C for two minutes**.**Genotype analysis for G460 A point mutation at exon 7 was carried out by PCR assay with an annealing temperature of 59°C for two minutes .

Statistical Analysis

Statistical analyses were done by using the SPSS version 22.0 for Windows, (SPSS Inc., Chicago, IL) [(6)].Genotype frequencies were tested using a Two-Sample Student's t-test to determine the difference in allele frequencies proportion in TPMT

Results

The study included 79 ALL patients newly diagnosed including (30 girls , 38.0%), and (49 boys ,(63.0%) .The three most common inactive alleles of the TPMT gene (TPMT*3A, TPMT*3B, and TPMT*3C) were sought. The TPMT*3A mutant allele was found in 18 patients with allele frequency of (22.8 %), while TPMT*3C mutant allele was detected in 5 children with allele frequency of 6.3% . But TPMT*3B mutant allele was not detected in the sample population as shown in table 1

Table 1: Genotype frequencies of TPMT variation in a sample of 79 ALL patients

Allele	SNP Position	rs	Amino acid Substitution	N	Frequency %
TPMT*1	Wild-type			56	70.9%
TPMT*3A	G460A A719G	1800460 1142345	Ala 154 Thr Tyr 240 Cyc	18	22.8%
TPMT*3B	G460A	1800460	Ala 154 Thr	0	0.00
TPMT*3C	A719G	1142345	Tyr 240 Cyc	5	6.3%
Total 79					

Result of digestion with allele-specific multiplex-PCR analysis for TPMT gene (TPMT*3A, TPMT*3B and TPMT*3C)) including 245

bp band for the wild-type of TPMT*1 as shown in fig 1 , for the (TPMT*3C) genotype 273 pb and two bands 273 and 338 pb for TPMT*3A as shown in fig.2.

Figure 1 product of TPMT gene polymorphism analyzed by agarose gel electrophoresis.

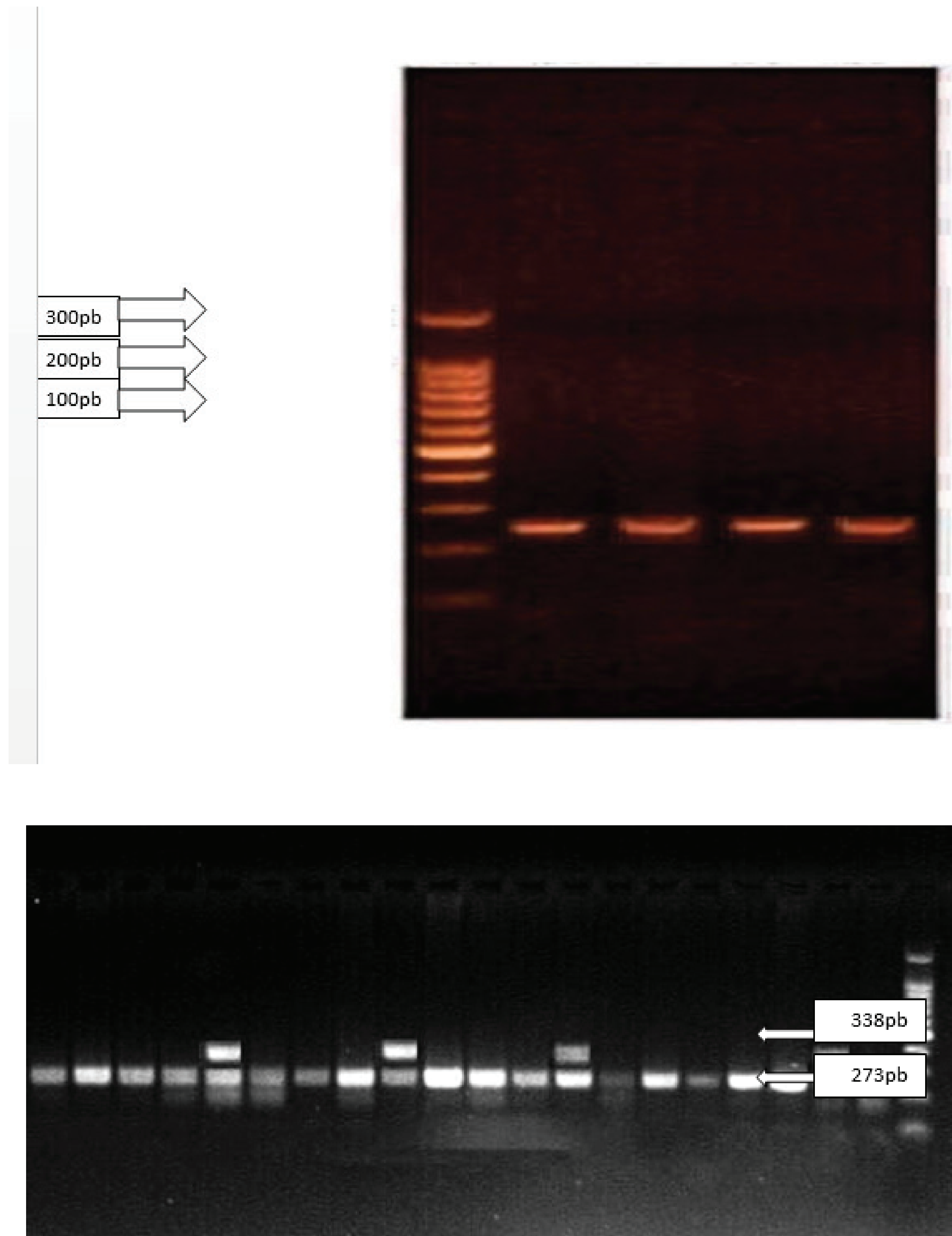

Figure 1. product of TPMT gene polymorphism analyzed by agarose gel electrophoresis .two fragments of 338 and 273 bp corresponds to TPMT*3A polymorphism, a single fragment of 273 bp corresponds to TPMT*3C polymorphism

Discussion

TPMT is one of the best examples of the application of pharmacogenetics to clinical practice involving the genetic polymorphism. Treating TPMT-deficient patients with standard doses of mercaptopurine (6MP), thioguanine or azathioprine can be fatal (6) , (7) such patients can be successfully treated, without severe toxicity, if the dose is properly adjusted (8). For this reason this study dealt with ALL patient which are newly diagnosed. Clinically it is well-known that patients with TPMT wild-type of non genetic factors (are still risk for thiopurine related hematotoxicity (9). and even after consideration of non-genetic factors (e.g. concomitant medication with the XO inhibitor allopurinol or viral infection) underlying mechanisms are so far unknown to completely explain thiopurine-related hematotoxicity (10). This study revealed that the prevalence of TMPT genetic polymorphism was higher in boys than in girls. There were 23 patients carrying TPMT mutant alleles (3A,3C) ; 10 out of 30 girl (33.3%) and 8 out of 49 (16.3%) have TPMT*3A and 1out of 30 (3.3%) and 4 out of 49 (8.1%) have TPMT*3C , the correlation between gender and the polymorphism was not statistically significant as p-value 0.23. This result is similar to the Korean study and a study on Bulgarian population which showed that there was a significant gender-related difference in TPMT enzyme activity (11, 12).

The most common variants are reported were *3A,*3B, and *3C all involving G460A and/or A719G .(13, 14) So, the present study focused on the detection of TPMT*3A and TPMT*3B and TPMT*3C alleles were detected in 23 patient out of 79ALL pediatric patients .TPMT*3A was detected in 18 patients with allele frequency of (22.8 %), while TPMT*3C mutant allele was detected in 5 children with allele frequency of (6.3%) . on other hand TPMT*3B mutant allele was not detected in the sample population , these findings were similar to studies study in Libyan (15) and other study in Iraq (16) . This differs from the findings among black Africans such as the Ghanaian and Kenyan populations, among whom only TPMT*3C was detected (9)

Furthermore, In Iraqi population, it was found that TPMT*3A mutant allele had a higher frequency than TPMT*3C mutant allele contribution to the overall frequency, 22.8% and 6.3%respectively. These outcomes are similar to other researches on the Caucasian population in which the TPMT*3A is the dominant allele as compared with TPMT*3C mutant allele [11].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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