ASSOCIATION BETWEEN TPMT GENOTYPE AND PHENOTYPE IN IRAQI CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

Esraa Ali Kadhum¹, Hasanein H.Ghali², Manal K Rasheed³
¹College of medicine, University of Baghdad, Baghdad, Iraq.  
²College of medicine, University of Baghdad, Baghdad, Iraq.  
³College of medicine, University of Baghdad, Baghdad, Iraq

ABSTRACT

Background: It’s cancer of blood cells owing to an increase in the number of white blood cells, they gather out the red blood cells and platelets that needed for the healthy body, and all those additional white blood cells cause problems. For this reason that they don’t work right.

Aim: To identify the level of TPMT activity and the most common TPMT polymorphism (TPMT*3A, TPMT*3B and TPMT*3C) and its frequencies in a sample of Iraqi ALL pediatric patients.

Materials and Methods: A cross sectional study was performed among 79 patients with Acute lymphoblastic leukemia. Genotyping for (*3A, *3B, *3C) types was performed by the allele-specific multiplex-PCR analysis method and TPMT activity in the serum was measured by using Enzyme-Linked Immunosorbent Assay Technique (ELISA).

Results: The mean of enzyme activity of each mutant allele was 4.95 ± 0.4, the independent t-test was calculated and indicate a statistically significant differences in mean enzyme activity level between wiled type allele and both mutant alleles as p-value was 0.05. Result indicates no significant differences between mean enzyme activity levels in both gene types according to gender, as p-value was 0.18 and 0.14.

Conclusion: TMPT genotyping and phenotype measurement is an essential tool to reduce the cytotoxic effects of the anti-cancer drug 6-MP in pediatric ALL treatment course. Keywords: 6-Mercaptopurine, Acute lymphoblastic leukemia, neutropenia, drug toxicity

I. INTRODUCTION

Acute lymphoblastic leukemia (ALL) a malignant disease in bone marrow wherein 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 gathering out the red blood cells and platelets that needed for the healthy body, and all those additional white blood cells cause problems. For the reason that they don’t work right. Mercaptopurine (6-MP) It is a purine antimetabolite, and it is frequently associated with life threatening myelosuppression. The TPMT enzyme has a molecular mass of 28 kDA and contains 246 amino acid residues. The enzyme is not metal-dependent and is present in most tissues, such as heart, blood cells, placenta, pancreas, and intestine TPMT enzyme activity varies in individual humans. (R.M. et al., 2010).

TPMT activity was significantly higher in blood samples of ALL patients on long-term 6-MP treatment compared with controls. (Lennard L et al., 1987). Changes in the TPMT gene cause TPMT deficiency, which is a reduction in the activity of the TPMT enzyme. Without enough of this enzyme, the body cannot "turn off" thiopurine drugs by metabolizing them into inactive compounds. The drugs stay in the body longer and continue to destroy cells unchecked, which leads to bone marrow damage (hematopoietic toxicity). This damage causes myelosuppression, which is an inability of the bone marrow to make enough red blood cells, white blood cells, and platelets. (Cooper SC et al., 2008), (Fotoohi AK et al., 2010), (Lennard L et al., 2014). TPMT was the first pharmacogene that showed a substantial association with 6-MP maximum tolerated dose and 6-MP related toxicities leading to the implementation of TPMT (Relling et al., 2013). Thiopurine methyltransferase or thiopurine S-methyltransferase
(TPMT) catalyzes S-methylation of thiopurine prodrugs such as mercaptopurine, azathioprine, and thioguanine to inactive metabolites (Yogita A et al., 2017)

II. MATERIALS AND METHODS

This a cross-sectional study included 79 patients with Acute Lymphoblastic Leukemia. The sample constituted 49 boys and 30 girls of the age group between 1.80-15.60 years, all of them were 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.

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 four weeks) 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 by allele-specific multiplex-PCR analysis method. The primer sequences were obtained (Nawar et al., 2019):

- 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’-GCTTTAACCACCGTCTCTG-3’
- TPMT*3C F- 5’-AAGTGTGGGATTACAGGTG-3’ and R- 5’-TCCTCAAAAAACATGTCAGTG-3’

Amplification was performed in a total volume of 23 μl which 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.

TPMT Phenotype

Two mL of peripheral blood was obtained from all patients’ in-plane tube and was clotted at room temperature. It was then centrifuged at 3600 rpm for 10 minutes. The obtained sera were frozen at -20°C in Eppendorf safe-lock tubes until used. This assay was performed using the TPMT ELISA Kit provided by MyBioSource, USA, with Sandwich-ELISA plates that have been pre-coated with an antibody specific to the TPMT enzyme.

III. RESULTS

The mean enzyme activity for all enrolled patient was 49.67 ± 131.26 ng/ ml, 56 of participant have TPMT *1 the wild type with enzyme range of activity 6.82 - 854.55 ng/ml and mean of 68.03 ± 20.37 ng/ml. 18 patient have TPMT 3A allele, range of enzyme activity 1.01 - 6.82 ng/ml and mean was 4.70 ± 0.44, 5 patient have TPMT 3C allele with range of enzyme activity 3.12 - 8.52 ng/ml and mean of 5.88 ± 0.90. The mean of enzyme activity of each mutant allele was 4.95 ± 0.4, the independent t-test was calculated and indicate a statistical significant differences in mean enzyme activity level between wild type allele and both mutant alleles as p-value was 0.05.

Table 1: enzyme activity level in each gene type

<table>
<thead>
<tr>
<th>SNP</th>
<th>N</th>
<th>Range of activity</th>
<th>Mean ±SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>79</td>
<td>1.01 - 854.55</td>
<td>49.67 ± 131.26</td>
<td>0.05*</td>
</tr>
<tr>
<td>TPMT*1</td>
<td>56</td>
<td>6.82 - 854.55</td>
<td>68.03 ± 20.37</td>
<td></td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>18</td>
<td>1.01 - 6.82</td>
<td>4.70 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>TPMT *3C</td>
<td>5</td>
<td>3.12 - 8.52</td>
<td>5.88 ± 0.90</td>
<td></td>
</tr>
</tbody>
</table>
Mutant gene (3A + 3C) 23 1.01 - 8.5 4.95 ± 0.4

Result indicates no significant differences between mean enzyme activity levels in both gene types according to gender, as p-value was 0.18 and 0.14. as shown in table 2

<table>
<thead>
<tr>
<th></th>
<th>Girl</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>19</td>
<td>11</td>
<td>37</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td>40.73 ± 20.05</td>
<td>4.88 ± 0.59</td>
<td>82.05 ± 28.99</td>
<td>5.02 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.18</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: mean enzyme activity level in both gene types according to gender

IV. DISCUSSION

Based on several clinical studies and various disease entities (e.g. ALL in childhood, Inflammatory bowel disease, autoimmune diseases) (Moon W et al., 2016), (pretreatment determination of the TPMT phenotype and subsequent pharmacogenetically-guided dosing of thiopurines, at least in TPMT deficient individuals, is recommended in routine clinical practice before commencing therapy (Relling MV et al., 2013) . Very recently a landmark randomized clinical trial strongly corroborates the clinical utility of upfront genetic testing for TPMT in patients treated with thiopurines to avoid hemotoxicity (Coenen MJ et al., 2015). It is recommended for measuring both TPMT activity and genotyping methods for the diagnosis of TPMT deficiency in risk individuals to reach safe and successful ALL treatment protocol. (Zielinska et al., 2016) TPMT activity measurement by ELISA assay would provide an easy and fast method for the patients who are intolerant to 6-MP therapy to identify the proper dose helping reduce drug-related toxicity, while genotype analysis may be more accurate for determining the actual TPMT levels [Samochatova et al., 2009]. Patients with full TPMT activity can be treated with a standard dose of the 6-MP drug from the beginning but risky patients with low TPMT activity are at risk of haemopoietic toxicity, myelosuppression or even death especially when they are given standard dose of thiopurine medication (Relling MV et al., 2011),( van Egmond R et al., 2013) .Those risky patients can be treated with a lower dose of 6-MP to reduce drug toxicity (Zeglam et al. 2015).

In this study the TPMT enzymatic activity of the patients in wild type group was found to be higher than mutant allele group (6.82 - 854.55 ng/ml versus 1.01-8.5ng/ml) as see in table 1 these result are inconsistent with (Nawar et al.,2019). The explanation for differences is that they might be considered as non-tolerant patients because of reasons other than TPMT genetic variation; such as they might have another TPMT mutant gene(s) that not studied in the present study, or might be due to non-genetic factors. This study indicated that there was a statistically significant differences in mean enzyme activity level between wiled type allele and both mutant alleles as p-value was 0.05 as see in table 1. This result is similar to a cohort consists of 245 children of European ancestry (Riin Tamm et al., 2017) .In Iraqi population, it was found that enzyme activity of all ALL patient (79)was only 6 (7.6%) patient have low enzyme activity, while all the other participant have normal activity (92.4% ) These results are archived with ( Schaeffeler E et al., 2003) , (Stanulla M et al., 2009).

REFERENCES


