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The effect of methylenetetrahydrofolate reductase polymorphisms on the methotrexate toxicity in children with acute lymphoblastic leukemia

Ebru Yılmaz[1], Alper Özcan[1], Veysel Gök[1], Musa Karakükcü[1,2], Ekrem Ünal[1,3,4]

[1]Erciyes University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology Oncology & Hematopoietic Stem Cell Transplant Unit, Kayseri, Turkey
[2]Erciyes University, Betul-Ziya Eren Genome and Stem Cell Center, Department of Molecular Biology & Genetic, Kayseri, Turkey·
[3]Erciyes University, Medical Sciences Institution, Department of Stem Cell Sciences, Kayseri, Turkey·
[4]Erciyes University, Health Science Institution, Department of Blood Banking and Transfusion Medicine, Kayseri, Turkey

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ABSTRACT

Aim: The enzyme of methylenetetrahydrofolate reductase (MTHFR) is fundamental for folate metabolism and has two common polymorphisms (C677T and A1298G). Methotrexate, which interrupts folate metabolism, is one of the backbone drugs of pediatric acute lymphoblastic leukemia (ALL). Methotrexate inhibits the synthesis of DNA replication.

Material and Method: In this study, we aimed to investigate the relationship between polymorphisms of the MTHFR gene and methotrexate toxicity. 85 children with newly diagnosed ALL were enrolled in the study. MTHFR gene polymorphisms and the toxicities related to methotrexate were evaluated.

Result: A total of 85 (54 females and 31 males) children were diagnosed with ALL. The allele frequencies for the FRG polymorphisms were as follows: MTHFR 677 CC 47 (55.3%), CT 29 (34.1%, TT 9 (10.6%) . No significant differences were detected with respect to event-free survival or toxicity between wild-type and other MTHFR variants.

Conclusion: Clinicians must be vigilant about the pharmacogenetic features of the patients. This study reveals that personalized medicine is the next future of treating ALL.

Keywords: Children, methotrexate, methylenetetrahydrofolate reductase, polymorphism, toxicity

INTRODUCTION

Childhood acute lymphoblastic leukemia (ALL) is cured in approximately 80% of patients (1-3). ALL regimens consist of combination chemotherapy administered continuously over 2 to 3 years (1,2). Although there are new drugs and treatment modalities, the treatment of ALL is still multidrug chemotherapy furthermore outcome of ALL may be influenced by modest changes in drug dose or exposure (2,3). Thus, if the determinants of interpatient variability in drug pharmacodynamics were better defined, tailoring drug therapy based on these factors might further improve outcome.

Methotrexate (MTX) is an antifolate chemotherapeutic agent that has been widely used in the treatment of childhood ALL (1-5). High-dose MTX infusion (1 g/m²) is usually given in the consolidation treatment and maintenance chemotherapy in most regimens including weekly oral MTX doses. Drug-related toxicities seen after MTX treatments including toxicities of the gastrointestinal system, such as oral mucositis, as well as toxicities of the skin, central nervous system, hepatic, renal system, and bone marrow (4,5).

The enzyme methylene tetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, in the folic acid cycle, and is interrupted by MTX therapy. The pharmacokinetics and pharmacodynamics of this agent is known to be influenced, at least in part, by genetic variations (6,7). The most common MTHFR polymorphism C677T (are nonsynonymous amino acid changes) affect

Corresponding Author: Ekrem Ünal, ekremunal@erciyes.edu.tr
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MTHFR enzyme causing a reduction of its activity, altered distribution of intracellular folate metabolites and increased levels of blood homocysteine (6-9). As a result of this mutation, homozygotes have ~30% of the normal MTHFR enzyme activity, while heterozygotes have 60% of normal MTHFR activity, causing impaired remethylating of homocysteine to methionine and subsequent hyperhomocysteimia (6,7).

In clinical studies, homozygosity of the C677T polymorphism has been associated with increased toxicity after MTX therapy of patients with ALL (6,7). Several reports have described association between MTHFR variants and clinical outcome, toxicity and susceptibility to ALL especially in pediatric population (6).

The MTHFR C677T variant allele has also been associated with increased toxicity with MTX (6-9) Although informative, all of these studies have utilized relatively small and heterogeneous sample sets and have not concurrently analyzed toxicity risks. Therefore, we sought to test the impact of MTHFR polymorphisms on clinically relevant toxicity end points in a large homogenous sample set.

Based on prior reports, we also hypothesized that patients with MTHFR variant alleles could experience increased toxicity. This report evaluates the role of the MTHFR C677T polymorphisms on toxicity risk in children with ALL patients, all of whom received the same dose and schedule of MTX according to the national Turkish BFM protocol (10).

MATERIAL AND METHOD

This study was approved by the Erciyes University Clinical Researches Ethics Committee (Date: 07.11.2018, Decision No: 2018/552). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In the present study, we investigated the influence of concurrently administered chemotherapy and MTHFR polymorphisms on toxicity after medium dosage (MD) MTX in children with ALL.

Patients

Of the 85 children with newly diagnosed ALL treated according to the TRALL 2002 protocol, at the Erciyes University Gevher Nesibe Hospital, Kayseri, Turkey, between January 2002 and December 2008, were enrolled in the study. TRALL 2002 protocol is the Turkish version of the Berlin-Frankfurt-Münster (BFM) 95 study for ALL (10). The consolidation regimen of this protocol for the standard or intermediate risk groups consisted of four courses of medium-dose methotrexate (MD-MTX) (1000 mg/m²/dose) with intrathecal injections of MTX (fig 1). Patients received MD-MTX over 36 hours in the courses with uniform supportive treatments followed by each protocol. MD-MTX treatment was followed by leucovorin rescue (15 mg/m²), which was administered 48 hours after the start of the methotrexate infusion and every 6 hours thereafter, for a total of two or three doses. Routine assessment of the serum MTX levels is not necessary in this protocol.

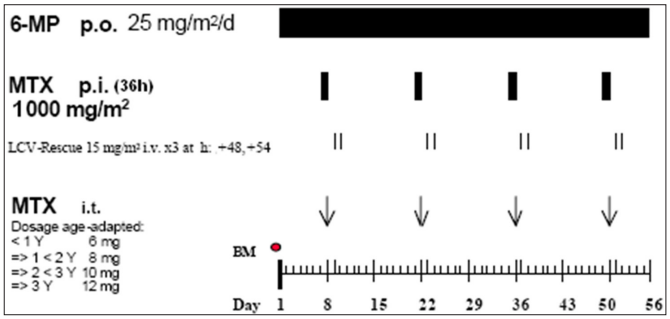

Figure 1. The schema of consolidation regimen of TRALL BFM protocol

Standard risk group was defined as age between 1 and 6 years at diagnosis, initial leukocyte count of<20 x 109L, non-T immunuphenotype, negative t(9;22) and t(4;11), and good prednisone response (Patients with an absolute blast count in the peripheral blood on day 8 of<1,000/mL are prednisone-good responders). Intermediate risk group was defined as age <1 or >6 years at diagnosis, initial WBC count of $>20\times10^9$/L, negative t(9;22) and t(4;11), and good prednisone response. High risk patients received different consolidation treatment but same maintenance chemotherapy.

Toxicities Evaluation

MD-MTX and maintenance therapy-related toxicity data were collected, retrospectively. Toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0, published by the National Cancer Institute.17.8.

The toxicity after each MD-MTX was registered in the period from the planned end of the MD-MTX course (72 h after the start of the infusion) and until the next course of MD-MTX was started. The next courses of chemotherapy were started 14 days after the MTX infusions.

The registration included nadir laboratory values [white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin level (Hb), platelet count (PLT), maximum plasma values of alanine aminotransferase (ALT)], occurrence of fever (hospitalization with temperature >38°C), and interruption of oral 6 mercaptopurine (6MP) and MTX maintenance treatment due to toxicity. Criteria for interruption of maintenance treatment included WBC $<1.0\times10^9$/L, platelet count $<50\times10^9$/L, occurrence of fever (temperature >38°C). Both treatment withdrawals and

delayed re-administrations (in courses where 6MP and oral low-dose MTX was deliberately paused during HD-MTX) were considered as interruptions of maintenance treatment.

Genotyping

The DNA extraction and Real-Time Polymerase Chain Reaction were performed as reported previously (11).

Statistical Analysis

The primary outcome of interest was development of toxicity in the subset of patients receiving homogeneous MD-MTX and maintenance chemotherapy. Correlation of MTHFR genotype with clinical characteristics such as age, sex, stage, therapy-related toxicity (hemoglobin (Hb), white blood cell (WBC), platelet, AST: Aspartate aminotransferase; ALT: Alanine aminotransferase total bilirubin, urea, creatinine levels and febrile neutropenic episodes), and outcome was performed using the $\chi 2$ or Fisher's exact test. The level of significance was performed to $p<0.05$ and data processing were evaluated with SPSS 15.0 statistical software for Windows.

The study was designed retrospectively, no written informed consent form was obtained from patients.

RESULTS

The files of 102 children with ALL were evaluated. 17 patients were not evaluable for toxicity during MD-MTX courses, because of high-risk leukemia sufferers being shifted to high-dose (HD) chemotherapy and stem-cell transplantation, and early relapse or death after induction chemotherapy and children with T lymphoblastic immunophenotyped ALL who received HD-MTX (5 g/m²/dose). Sixty-one patients were considered evaluable for toxicity of the MD-MTX chemotherapy; these patients were excluded from the study. Total 85 patients were evaluated for MTHFR genotype. Access was achieved to the data regarding 85 patients (54 female and 31 male) monitored with the diagnosis of ALL. According to TRALL BFM protocols, 28 of the patients were classified as standard risk group (SRG), 38 were medium risk group (MRG), 19 high risk group (HRG) leukemia. The baseline characteristics of the ALL patients are shown in **Table 1.**

Genotype and Allele Frequencies of MTHFR gene

The wild-type genotype (CC) was present in 47 patients (55.3%), heterozygous genotype (CT) in 29 patients (34.1%) and homozygous genotype (TT) in 9 patients (10.6%).

The scheduled dosage of oral 6MP was 40 mg/m² a day and that of oral MTX was 25 mg/m² once a week.. The patient characteristics and clinical data used in this study were collected retrospectively, from the patient's chats.

Table 1. Patients characteristics

Characteristics	Genotyped Patients (n=85) (%)
Age at diagnosis (years)(mean)	
1 - 6 years	52 (61.1)
< 1 or >6 years	33 (38.9)
Gender	
Male	54 (63.5)
Female	31 (36.5)
WBC count (x109) at diagnosis	
<10	40 (47.1)
>10	45 (52.9)
Risk groups	
SRG (Standard risk group)	28 (32.9)
IRG (Intermediate risk group)	38 (44.7)
HRG (High risk group)	19 (22.4)
Overall MTHFR 677C/T genotype	
CC	47 (55.3)
CT	29 (34.1)
TT	9 (10.6)
MTHFR 677C/T genotyping for maintenance therapy	51
CC	25 (49)
CT	21 (41.2)
TT	5 (9.8)
Duration of maintenance therapy (wk) (median)	8
Cumulative MTX doses of maintenance	3
Number of relapsed patients	8
Survival status	
Alive	73 (85.9)
Died	12 (14.1)
Median Follow-up (months) (median)	37

SRG: Standard risk group, IRG: Intermediate risk group, HRG: High risk group.

Survival Status

Twelve (14.q%) of the 85 children died during follow-up (median 37 months). No deaths occurred in patients However, there were no significant differences with respect to relapse rates, event free survival (EFS) between the groups with and without polymorphic variants of MTHFR gene polymorphisms (**Figures 2**).

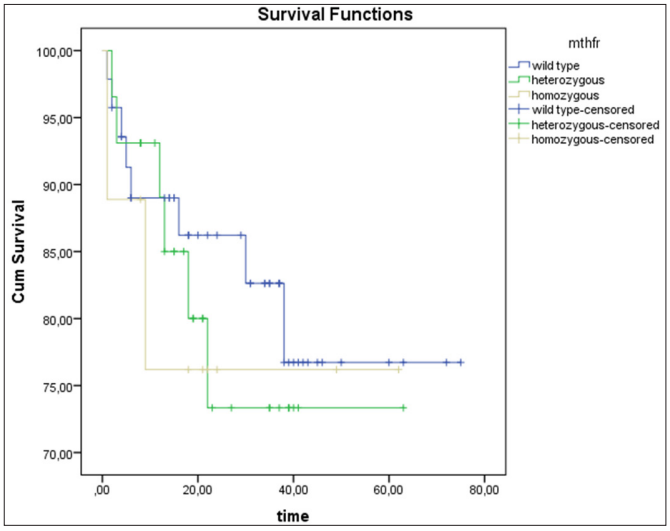

Figure 2. The event free survival of children with acute lymphoblastic leukemia

DISCUSSION

MTX is a backbone of consolidation and maintenance treatment for children with ALL (1-3). However, some patients cannot tolerate HD-MTX, and in these cases the treatment can cause toxicity and discontinuation of chemotherapy, which may increase relapse (5-7).

Maggdenberg et al. (5) reported that the association between genetic variations in genes involved in MTX pharmacodynamics (e.g. MTHFR) or pharmacokinetics and the development of mucositis. Moreover mucositis can impair oral intake of food and liquids and an impaired quality of life during therapy, often requiring dose reductions or cessations of treatment which can interfere with treatment efficacy. Yazıcıoğlu et al. (6) observed higher rate of renal toxicity in patients who carried the MTHFR 677CT/TT genotypes compared to wildtype MTHFR enzymes.

There are conflicting results regarding the roles of the MTHFR gene polymorphisms in leukemia prognosis (5-7). Some studies have indicated that these variants play protective roles, whereas others have shown that they are linked to increased rates of relapse and drug resistance (1-6). In accord with these data, we observed no significant differences in EFS between patients with or without polymorphic variants of MTHFR and other enzymes, and this finding supports the effect of HD-MTX in our study.

On the other hand, MTHFR variants were reported to be associated with arterial, venous thrombosis. Of the determined underlying causes of thrombosis, the polymorphisms at the genes of the enzymes involved in homocysteine metabolism may confer an increased risk for thrombosis by causing hyperhomocysteinemia. Population-based studies revealed that neither of the risk alleles (MTHFR C677T and MTHFR A1298C) of the homocysteine metabolism-related genes alone increased the risk for the occurrence of stroke (12) Kenet et al. (13) conducted a systematic search of various electronic databases for studies published from 1970 to 2009 and found that hyperhomocysteinemia but not MTHFR polymorphisms identifies individuals at increased thrombotic risk. Several studies emphasized that MTHFR polymorphisms without hyperhomocysteinemia do not have any importance on thrombosis (8,9,12,13).

The small sample size of our study population is the main limitation of the present findings. We observed no significant differences between wild-type and other MTHFR variants with respect to toxicity or relapse rate, but further investigations with larger patient numbers are needed.

CONCLUSION

MTHFR C677T polymorphisms in Turkish children with ALL are similar to those reported for other Caucasian populations. Our results indicate that HD-MTX can be tolerated by leukemic children with some polymorphic variants and thus it may prevent future risk of leukemic relapse.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Erciyes University Clinical Researches Ethics Committee (Date: 07.11.2018, Decision No: 2018/552).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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REFERENCES

1. Inaba H, Pui CH. Advances in the diagnosis and treatment of pediatric acute lymphoblastic leukemia. J Clin Med 2021; 10: 1926.
2. Inaba H, Mullighan CG. Pediatric acute lymphoblastic leukemia. Haematologica 2020; 105: 2524-39.
3. Micallef B, Nisticò R, Sarac SB, ey al. The changing landscape of treatment options in childhood acute lymphoblastic leukaemia. Drug Discov Today 2022; 27: 1483-94.
4. Koźmiński P, Halik PK, Chesori R, Gniazdowska E. Overview of dual-acting drug methotrexate in different neurological diseases, autoimmune pathologies and cancers. Int J Mol Sci 2020; 21: 3483.
5. Maagdenberg H, Oosterom N, Zanen J, et al. Genetic variants associated with methotrexate-induced mucositis in cancer treatment: a systematic review and meta-analysis. Crit Rev Oncol Hematol 2021; 161: 103312.
6. Yazıcıoğlu B, Kaya Z, Güntekin Ergun S, et al. Influence of folate-related gene polymorphisms on high-dose methotrexate-related toxicity and prognosis in Turkish children with acute lymphoblastic leukemia. Turk J Haematol 2017; 34: 143-50.
7. Frikha R, Rebai T, Lobna BM, et al. Comprehensive analysis of Methylenetetrahydrofolate reductase C677T in younger acute lymphoblastic leukemia patients: a single-center experience. J Oncol Pharm Pract 2019; 25: 1182-6.

8. Torun YA, Patiroglu T, Ozdemir MA, Ozkul Y, Ekici A, Karakukcu M. Inherited prothrombotic risk factors in Turkish children with acute lymphoblastic leukemia: significance of concomitant genetic mutation. Clin Appl Thromb Hemost 2012; 18: 218-21.
9. Unal E, Mutlu FT, Karakukcu M. The importance of MTHFR polymorphisms in pediatric cerebral stroke. Childs Nerv Syst 2012; 28: 13.
10. Yuksel Soycan L. BFM-TR ALL 2000: first Turkish multicentric study in the treatment of pediatric acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2007; 29: 21.
11. Ozkul Y, Evereklioglu C, Borlu M, et al. 5,10-Methylenetetrahydrofolate reductase C677T gene polymorphism in Behcet's patients with or without ocular involvement. Br J Ophthalmol 2005; 89: 1634-7.
12. Akar N, Akar E, Ozel D, Deda G, Sipahi T. Common mutations at the homocysteine metabolism pathway and pediatric stroke. Thromb Res 2001; 102: 115-20.
13. Kenet G, Lütkhoff LK, Albisetti M, et al. Impact of thrombophilia on risk of arterial ischemic stroke or cerebral sinovenous thrombosis in neonates and children: a systematic review and meta-analysis of observational studies. Circulation 2010; 121: 1838-47.