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Relationship Between Plasma Methotrexate Level At 42 Hours And Toxicity After High-Dose Methotrexate In Children With Acute Lymphoblastic Leukemia: A Prospective Cohort Study

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ABSTRACT

Background : Despite its efficacy in treating acute lymphoblastic leukemia, high-dose methotrexate (HDMTX) can cause serious side effects. Methotrexate monitoring (MTX) is challenging in resource-limited settings.

Objective: The aim of this study was to compare the toxicities of different doses of HDMTX and investigate the relationship between plasma MTX levels at 42 hours from the start of HDMTX infusion (MTX_{42}) and possible toxicities.

Patients and Methods: This prospective study consecutively enrolled 30 children with acute lymphoblastic leukemia in Egypt between May 2018 and July 2020. Patients were stratified into two risk groups according to the TOTAL therapy study XV protocol. MTX was administered intravenously as a 24-hr infusion (low-risk: 2.5 g/m^2, standard/high-risk: 5 g/m^2). The Common Terminology Criteria for Adverse Events v.4.03 was used to report toxicity. MTX_{42} levels were estimated after the first HDMTX infusion.

Results: The most common toxicities were anemia and neutropenia in both groups. There was no statistically significant difference between the two groups regarding toxicities. Moreover, there was no significant difference between patients with and without different toxicities regarding MTX_{42} levels.

Conclusion: Higher MTX doses can be safely administered while applying proper supportive measures. Although plasma MTX level monitoring is recommended, MTX_{42} levels failed to predict different toxicities.

Keywords: Methotrexate ; Acute Lymphoblastic Leukemia; Children

INTRODUCTION

The most common cancer in children is acute lymphoblastic leukemia (ALL).[1] Much of the current focus is on maintaining high survival rates while limiting the long-term toxicities of treatment.[2] Anti-folate drugs were among the first antineoplastic agents. Methotrexate (4-amino-10-methylfolic acid, MTX) is an analog and antagonist of folic acid. Nowadays, high-dose methotrexate (HDMTX) is critical in the treatment of pediatric ALL. HDMTX was initially introduced to improve penetration into sanctuary sites such as the central nervous system (CNS) and testicular tissue and to overcome cellular resistance to MTX. However, patients receiving HDMTX can encounter severe and prolonged toxicities.[3,4]

After an HDMTX infusion with a fixed dose and duration, plasma concentrations can vary widely between patients and within a patient on different cycles.[5] It is still debatable whether plasma MTX levels following HDMTX should be used to predict the occurrence of adverse events.[6] Unfortunately, many centers in resource-limited settings either have no access to the test or resort to a limited sampling strategy at one specific time point.[7] Through this study, we compared the toxicity of different doses of HDMTX according to risk stratification (Low-risk: 2.5 g/m^2 versus standard/high-risk: 5 g/m^2) in children with ALL and investigated the relationship between MTX_{42} levels and possible toxic effects.

The study aimed to compare the toxicities of different doses of HDMTX and investigate the relationship between plasma MTX levels at 42 hours from the start of HDMTX infusion (MTX_{42}) and possible toxicities.

PATIENTS AND METHODS

The present prospective cohort study consecutively recruited 30 ALL children younger than 15 years at diagnosis admitted at the Pediatric Hematology/Oncology unit, Alexandria University Children's Hospital, Egypt, between May 2018 and July 2020. The study was approved by the ethics committee of the Faculty of Medicine, Alexandria University. Informed consent/assents were signed by the patient's legal guardians/patients. According to the TOTAL therapy study XV protocol,[8] patients were stratified into two risk groups (15 patients in the low-risk group and 15 patients in the standard/high-risk group).

Treatment regimen

All patients received the first HDMTX infusion in the consolidation phase. Pre-hydration intravenous (IV) fluids were administered at a rate of 150 $ml/m^2/hr$ for 12 hours before HDMTX (dextrose 0.45% normal saline, potassium chloride 20 mmol/L, and sodium bicarbonate 40 mEq/L). The urine pH was measured using a dipstick, and the HDMTX infusion was not started until the pH was ≥ 6.5. MTX was administered as a 24-hr IV infusion (low-risk: 2.5 g/m^2, standard/high-risk: 5 g/m^2), where 10% of the dose was administered

over one hour as a loading dose, and the remaining 90% was administered evenly during the subsequent 23 hours. IV hydration was maintained during the HDMTX infusion.

Triple intrathecal therapy with MTX, hydrocortisone, and cytarabine was given on the day of HDMTX plus daily oral 6-mercaptopurine at 50 mg/m^2/day for the two weeks of the cycle. HDMTX was administered, provided the listed criteria were fulfilled: absolute neutrophil count (ANC) ≥0.3 x10^{*9}/L, WBC ≥1.0 x10^{*9}/L, platelet count ≥50 x10^{*9}/L, alanine aminotransferase (ALT) <500 U/L, total bilirubin <34.2 µmol/L and, direct bilirubin <23.94 µmol/L. Any concurrent treatment with Trimethoprim-sulfamethoxazole was paused two days before and three days after the HDTMX infusion. Leucovorin (LV) 15 mg/m^2 IV for standard/high-risk patients and 10 mg/m^2 IV for low-risk patients was started 42 hours after the beginning of HDMTX infusion and repeated every 6 hours for a total of five doses.

Determination of plasma MTX concentration

Two milliliters (mL) of venous blood were withdrawn from each patient at 42 hours from the start of the HDMTX infusion (MTX_{42}) during the first cycle of the consolidation phase.[8] Plasma MTX levels were estimated using the ELISA technique (Human Methotrexate ELISA Kit, Sino Gene Clon Biotech Co., Ltd, China).

Toxicity criteria and monitoring

During the first consolidation cycle, patients were assessed daily for three days after the beginning of HDMTX infusion in the inpatient ward, then in the outpatient clinic weekly by a hematology specialist to detect possible MTX side effects until the next cycle. The National Cancer Institute Common Terminology Criteria for Adverse Events v.4.03 (CTCAE) score system was used for grading gastrointestinal, hematologic, and eye toxicities.[9] Neutropenia, anemia, thrombocytopenia, elevation of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin, and serum creatinine were categorized in the laboratory category.[9] The length of additional hospitalization and delay of subsequent chemotherapy cycles due to MTX toxicity were documented.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). For comparing categorical variables, a Chi-square test was used, with Fisher's Exact test or Monte Carlo correction when needed. For abnormally distributed quantitative variables, a comparison between two groups was made using the Mann-Whitney test. The significance of the obtained results was judged at the 5% level.

RESULTS

Thirty children who received 30 HDMTX cycles were enrolled in the study. The demographic characteristics and clinical features of the children are presented in **(Table 1**). The comparison of the observed toxicities (clinical and laboratory) according to risk stratification. There was no mortality attributed to MTX toxicity. Moreover, no respiratory, neurologic, or skin toxicity were observed in the present study (**Tables 2 and 3**).

Regarding MTX_{42} levels, they were not significantly different irrespective of patients' risk stratification **(Figure 1a)**. The relations between MTX_{42} levels and common toxicities

were analyzed in total patients. Toxicity grades 1& 2 were considered mild toxicities, while grades 3 & 4 were considered severe.[6] Regarding anemia and thrombocytopenia, grade ≥3 was used for analysis. There was no significant difference in MTX_{42} levels between patients with and without the following toxicities: oral mucositis, vomiting, febrile neutropenia, anemia grade 3, thrombocytopenia grade 3&4, increased ALT, or increased serum creatinine. **Figure 1 (b-d)** and **Figure 2 (a-d)** illustrate the MTX_{42} levels among patients with and without different toxicities. The comparison between the low and standard/high-risk groups according to the length of additional hospitalization and the delay of subsequent chemotherapy cycles due to MTX toxicity is shown in **(Table 4)**.

Table 1. Demographic data of the studied patients:

Patients' data	**Low-risk (n = 15)**	**Standard/High-risk (n = 15)**
	N (%)	
Sex		
Male	5 (33.3)	10 (66.7)
Female	10 (66.7)	5 (33.3)
ALL subtype		
B-ALL	15 (100.0)	11 (73.3)
T-ALL	0 (0.0)	4 (26.7)
Age at diagnosis (years)		
Median (IQR) Min. – Max.	4.0 (3 – 5) 2.3 – 6.8	5.3 (3.4 – 6.6) 2.3 – 13.3

ALL: acute lymphoblastic leukemia, IQR: interquartile range, Min. – Max.: minimum – maximum, N (%): number (percent).

Table 2. Comparison of observed clinical toxicities according to risk stratification.

Observed toxicities (Clinical)	**Low-risk (n = 15)**	**Standard/High-risk (n = 15)**	**p**-value
	N (%)		
Gastrointestinal toxicities			
Vomiting			
None	10 (66.7)	7 (46.7)	^{MC}p=0.250
Grade 1& 2	5 (33.3)	5 (33.3)	

Grade 3	0 (0)	3 (20)	
Oral mucositis			
None	4 (26.7)	8 (53.3)	^{MC}p=0.148
Grade 1& 2	7 (46.7)	2 (13.3)	
Grade 3	4 (26.7)	5 (33.3)	
Diarrhea	2 (13.3)	2 (13.3)	^{FE}p=1.000
Abdominal pain	2 (13.3)	3 (20.0)	^{FE}p=1.000
Gastric hemorrhage (Grade 2)	0 (0.0)	1 (6.7)	^{FE}p=1.000
Hematologic toxicity			
Febrile neutropenia (Grade 3)	8 (53.3)	8 (53.3)	1.000
Eye toxicity			
Conjunctivitis (Grade 2)	2 (13.3)	1 (6.7)	^{FE}p=1.000

The non-mentioned grades of toxicity in the table were not observed in the present study. FE: Fisher Exact correction for Chi-square test, MC: Monte Carlo correction for Chi-square test.

Table 3. Comparison of observed laboratory toxicities according to risk stratification.

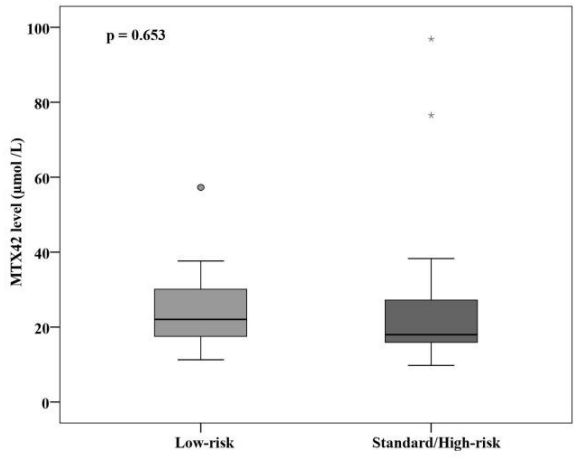
Observed toxicities (Laboratory)	**Low-risk (n = 15)**	**Standard/high-risk (n = 15)**	**p-value**
	N (%)		
Neutropenia			
None	2 (13.3)	1 (6.7)	^{MC}p=0.479
Grade 1 & 2	3 (20)	1 (6.7)	
Grade 3 & 4	10 (66.7)	13 (86.7)	
Anemia			
Grade 1 & 2	10 (66.7)	7 (46.7)	0.269
Grade 3	5 (33.3)	8 (53.3)	
Thrombocytopenia			
None	11 (73.3)	7 (46.7)	^{MC}p=0.135
Grade 1 & 2	0 (0)	4 (26.7)	
Grade 3 & 4	4 (26.7)	4 (26.7)	
Increased ALT			
None	11 (73.3)	7 (46.7)	^{MC}p=0.286
Grade 1 & 2	2 (13.3)	6 (40.0)	

Grade 3	2 (13.3)	2 (13.3)	
Increased AST			
None	11 (73.3)	9 (60.0)	^{MC}p=0.831
Grade 1 & 2	3 (20.0)	5 (33.3)	
Grade 3	1 (6.7)	1 (6.7)	
Increased total bilirubin			
None	13 (86.7)	11 (73.3)	^{FE}p=0.651
Grade 1 & 2	2 (13.3)	4 (26.7)	
Increased serum creatinine			
None	12 (80)	10 (66.7)	^{MC}p=0.684
Grade 1 & 2	3 (20)	4 (26.7)	
Grade 3	0 (0)	1 (6.7)	

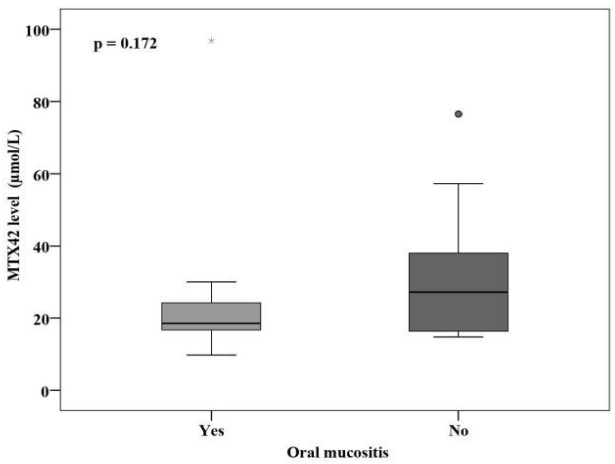
The non-mentioned grades of toxicity in the table were not observed in the present study.

ALT: alanine aminotransferase, AST: aspartate aminotransferase, FE: Fisher Exact correction for Chi-square test, MC: Monte Carlo correction for Chi-square test.

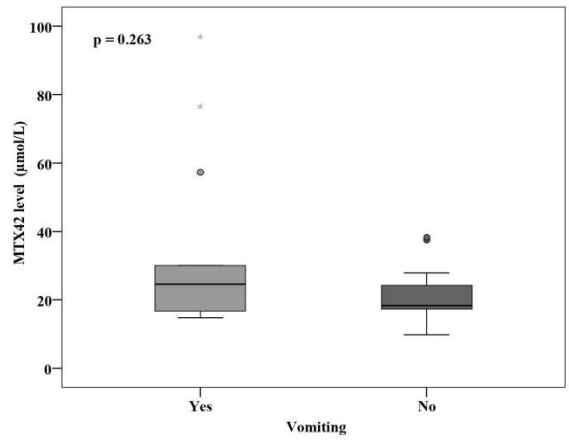
(a)



(b)



(c)



(d)

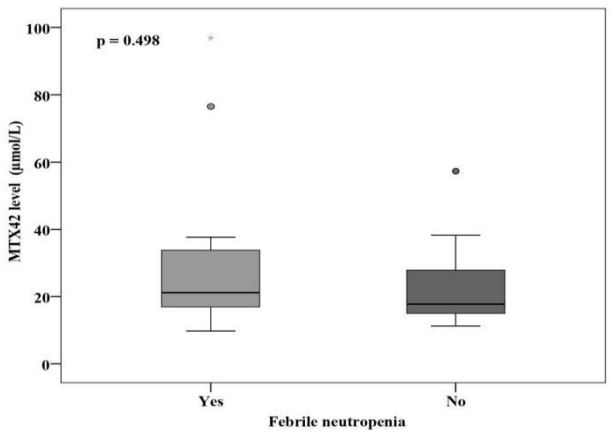
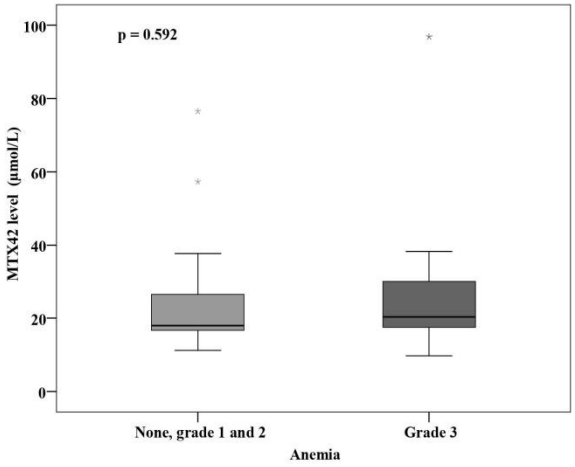
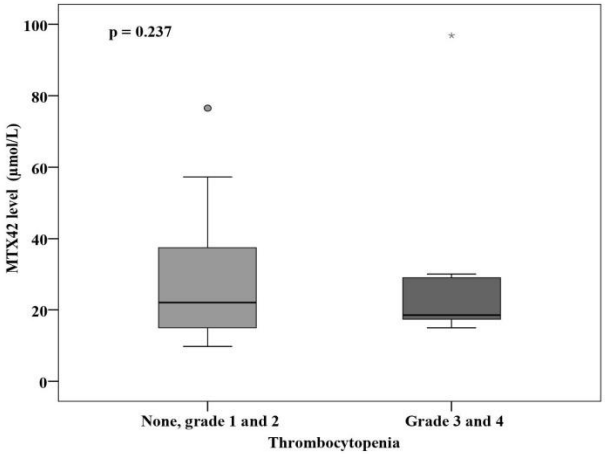


Figure (1): Comparison of the MTX42 level after the first HDMTX cycle according to the commonly observed clinical toxicities in all patients. a: in relation to the risk stratification, b: in relation to oral mucositis, c: in relation to vomiting, d: in relation to febrile neutropenia.

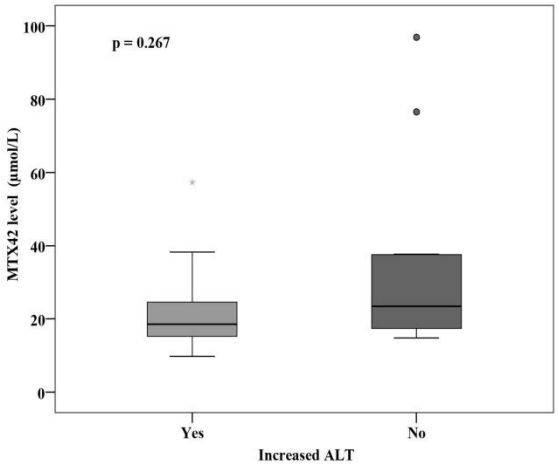
(a)



(b)



(c)



(d)

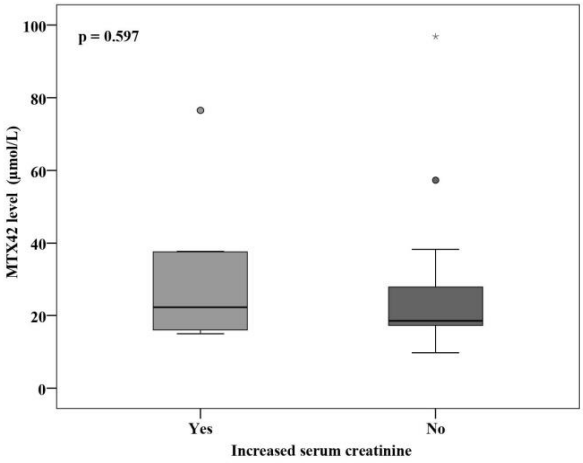


Figure (2): Comparison of the MTX42 level after the first HDMTX cycle according to the commonly observed laboratory toxicities in all patients. a: in relation to anemia, b: in relation to thrombocytopenia, c: in relation to increased ALT, d: in relation to increased serum creatinine.

Table 4. Comparison between the low-risk and standard/high-risk groups according to additional hospitalization and delay of subsequent chemotherapy cycles.

	Low-risk (n = 15)	**Standard/High-risk (n = 15)**	**p-** value
Length of additional hospitalization* (days)	**(n = 11)**	**(n = 5)**	
Median (IQR)	4.0 (2.5 – 7.5)	10.0 (6.0 – 10.0)	0.069
Min. – Max.	2.0 – 10.0	6.0 – 12.0	
Delay of subsequent Chemotherapy cycles # (days)	**(n = 6)**	**(n = 7)**	
Median (IQR)	6.0 (5.0 – 6.0)	4.0 (2.5 – 10.0)	0.731
Min. – Max.	5.0 – 7.0	2.0 – 19.0	

*Additional hospitalization attributed to MTX toxicity.

#Delay of subsequent chemotherapy cycles due to MTX toxicity, IQR: Interquartile range, Min.-Max.: minimum-maximum, n: number of patients, p-value for Mann Whitney test.

DISCUSSION

High-dose methotrexate can decrease the rate of relapse, prolong event-free survival, and suppress the development of CNS leukemia in ALL.[10] Despite its efficacy, HDMTX

therapy may cause substantial toxicity as myelosuppression, acute liver toxicity, nephrotoxicity, mucositis, and neurotoxicity.[11]

In the present study, we compared the toxicity of different doses of HDMTX during the consolidation phase. Anemia and neutropenia were the most common toxicities in both groups. This matches other studies,[6,7,12,13] which reported the most common toxicity of HDMTX to be myelosuppression but with varying incidences. We have reported relatively higher toxicity rates than reported by other studies.[7,13] We also detected a delayed MTX elimination in all patients, defined as MTX_{42} level >1 µmol/L after the first HDMTX infusion.[14] This could be a possible explanation for higher toxicity rates. However, MTX_{42} levels were not statistically different between patients with and without common toxicities.

Compared to other studies,[13,15] we have reported high MTX_{42} levels in both risk groups. These high levels may be due to withdrawing all samples in the first consolidation cycle, which is expected to show the highest levels. Nakano et al.[16] reported the first HDMTX infusion as a risk factor for delayed MTX elimination caused by low folate levels before therapy. Similarly, Mandal et al.[12] reported high levels (≥10 to <200 µmol/L) in 78% of 5 g/m^2 HDMTX cycles administered to children with ALL. However, they observed that delayed MTX elimination after the first HDMTX infusion did not imply that it would occur with subsequent infusions. Other factors that might contribute to delayed MTX elimination, including the use of drugs that impair MTX clearance and the presence of extravascular fluid collections as ascites, pleural effusions, and intracranial fluid collection, were not reported with any patient throughout our study.

According to the TOTAL therapy study XV protocol,[8] additional MTX levels should be obtained in patients showing high MTX_{42} levels (>0.5 µmol/L), to adjust leucovorin rescue. However, this was not feasible in our center due to the non-availability of the test, the long turnaround time when samples were sent to another laboratory, and the lack of enough samples from a single center, making the testing more costly.

According to the results of our study, there was no significant relationship between the MTX_{42} levels and the occurrence of toxicities. Other studies showed conflicting results regarding this issue. Özdemir et al.[11] found MTX_{42} levels to be unrelated to hematological and hepatic toxicities in children with ALL. Similarly, Kapoor et al.[17] observed no statistically significant relation between MTX_{42} levels and different toxicities (mucositis, neutropenia, thrombocytopenia, fever, and elevated transaminases). On the other hand, Tsurusawa et al.[18] reported that plasma MTX levels at 48 and 72 hours were not reliable predictors for toxicity except for nephrotoxicity in childhood B-Non Hodgkin lymphoma (B-NHL).

Anticipation of toxicity is sometimes challenging, considering the wide inter-patient variations in the metabolism of MTX based on unique individual's pharmacogenetic profile.[19,20] In the absence of MTX level testing, strict monitoring of urine pH and output, serum creatinine, and twice-daily examination of mucosal membranes are done as an alternative strategy for the safe administration of HDMTX in many centers.[21] Another suggested approach was the administration of HDMTX at full doses, with extended hydration and additional leucovorin doses to minimize toxicity,[7] with a reported risk of reducing the HDMTX efficacy.[22]

This study had several limitations; first, the small sample size, especially when divided into two groups for comparison. Second, the monitoring of MTX levels was done

only for the first consolidation cycle. Finally, extended MTX level monitoring was not performed to assess how long the patients have been exposed to potentially toxic levels. Nevertheless, our study highlights that despite the delayed MTX elimination encountered, HDMTX-related toxicities were manageable.

CONCLUSION:

The HDMTX toxicity profile was comparable irrespective of the dose of MTX (2.5 g/m^2 versus 5 g/m^2). This may suggest that higher MTX doses can be safely administered while applying proper supportive measures. Although plasma MTX level monitoring is recommended, MTX_{42} levels failed to show an association with different toxicities.

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