

Evaluation of Hepatic Complications for High Dose Methotrexate in Children: 10 Years after Treatment of Malignancy

Maryam Shoaran¹, Azim Rezamand², Soheyla Ghari³, *Leila Vahedi⁴

¹Associate Professor, Specialist of Pediatric Gastroenterology and Liver, Pediatric Health Research Center, Tabriz University of Medical Sciences, Iran. ²Associate Professor, Specialist of Pediatric Oncology, Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. ³MD, Department of Pediatrics, Pediatric Health Research Center Tabriz University of Medical Sciences, Tabriz, Iran. ⁴Assistant Professor, MD-PhD of Medical Genetics, Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Abstract

Background

Methotrexate is a chemotherapy drug used in high doses to treat malignancies that can lead to serious liver damage, especially cirrhosis in a dose-dependent manner. We aimed to evaluate the hepatic complications, 10 years after treating children with hematologic malignancies by high-dose Methotrexate regimen.

Materials and Methods: In a descriptive-analytical study, all children with hematologic malignancies who received high-dose methotrexate for treating cancer and who were hospitalized in Tabriz Children's Hospital, Tabriz, Iran, from 2008 to 2009 were included. Demographic data were extracted from medical records. Liver enzyme tests, ultrasonography, as well as a Doppler ultrasound of the portal vein were performed for all participants in the Tabriz Children's Hospital. Data were analyzed using descriptive statistics. The Chi-Square test was used to examine the relationship between variables using SPSS software version 22.0.

Results: Out of 30 patients, 18 cases (60%) were boys and 23 (76.67%) of patients had Acute Lymphoblastic Leukemia. ALT, Total BILL, and Direct BILL levels were increased by 13.33%, 13.33%, and 10% of cases, respectively. AST, PT, and PTT levels were normal in all patients. The liver and spleen spans were larger than normal in 26.67% and 40% of cases, respectively and 5 patients (16.67%) had grade 1 fatty liver. Port vein diameter was normal in all patients.

Conclusion

Based on the results of the study, it is possible to consider the long-term safety of high dose Methotrexate in terms of the development of severe liver disease following treating malignancies in children. However, patient follow-up is recommended to identify complications for years after discontinuing treatment.

Key Words: Children, Hepatic complications, Liver cirrhosis, Malignancies, Methotrexate.

*Please cite this article as: Shoaran M, Rezamand A, Ghari S, Vahedi L. Evaluation of Hepatic Complications for High Dose Methotrexate in Children: 10 Years after Treatment of Malignancy. Int J Pediatr 2021; 9(2): 13059-66. DOI: [10.22038/IJP.2020.54376.4301](https://doi.org/10.22038/IJP.2020.54376.4301)

*Corresponding Author:

Leila Vahedi, Assistant Professor, MD-PhD of Medical Genetics, Liver and Gastrointestinal Diseases Research Center, Imam Reza Hospital, Gholghasht street, Tabriz University of Medical Sciences, Tabriz, Iran.

Email: vahedi.l49@gmail.com or vahedil@tbzmed.ac.ir

Received date: Aug.10, 2020; Accepted date: Nov. 12, 2020

1- INTRODUCTION

The liver is one of the most important body organs, performing important and vital functions (1). Liver cirrhosis is among important damage caused by the fibrosis and necrotic inflammation of the liver (2). Drug-Induced Liver Injury (DILI) is one of the important causes of liver damage, and methotrexate (MTX) is among the important drugs causing dose-dependent DILI (3, 4). MTX is a chemotherapeutic drug acting as an anti-folate metabolite mainly used in treating malignancies such as acute lymphoblastic leukemia (ALL). It also has an essential role in the treatment of autoimmune diseases such as psoriasis, rheumatoid arthritis, and Crohn's disease (5-7). Chemotherapy regimens containing MTX are divided into 3 groups as high-dose (HD), intermediate-dose (ID), and low-dose (LD). The first (i.e. HD-MTX) regimen is generally used to treat and control malignancies (8-10).

Among pediatric malignancies, ALL and lymphoma are the most common neoplasms. Children with these malignancies are treated with MTX. Today, the elevation of liver aminotransferases is a well-known side effect of MTX (8, 9, 11). In fact, the main concern about this drug is related to its effects in inducing histopathological changes such as fibrosis, and cirrhosis in the liver (6, 12). Generally, liver complications have been reported in 10% of MTX consumers (3). Symptoms range from a variety of mild manifestations such as an increase of liver aminotransferases in the blood to severe and fatal events such as cirrhosis and fibrosis (3). The risk factors exaggerating MTX-induced hepatotoxicity include a history of alcohol consumption, chronic hepatitis B and C infections, a family history of liver diseases, diabetes mellitus, and a history of exposure to hepatotoxic drugs and chemicals (6, 13). In the study of Bath *et al.*, advanced fibrosis

or cirrhosis was observed in 5% of patients who either received low dose MTX for a long period or carried one or more of the above-mentioned risk factors (6). Evaluation of long-term liver damages in patients receiving MTX helps to characterize drug-induced side effects, choose more appropriate and faster diagnostic and therapeutic modalities for these patients. Given the importance of the issue, we conducted this study to evaluate the hepatic complications of MTX in children with malignancy, admitted to the Tabriz Children's Hospital for children 10 years after treatment.

2- MATERIALS AND METHODS

2-1. Study design and population

Hepatic complications were evaluated 10 years after treating children with hematologic malignancies by HD-MTX regimen (doses ≥ 500 mg/m²) for 6 months. This study was performed on Azeri Turks, who are members of one of the largest ethnic groups in Iran (14).

2-2. Methods

In a descriptive-analytical study, the patients hospitalized during years 2008 to 2009 were reviewed using the census method. Variables included age, sex, ultrasound of liver and bile ducts, and Doppler ultrasound of portal vein, the level of AST, ALT, PT, PTT, Total Bilirubin, and Direct Bilirubin. The demographic information was collected from medical records, patients or their parents, through interviews.

2-3. Measuring

Liver function tests, liver and bile duct ultrasonography, and Doppler vein port ultrasound were performed after explaining the objectives of the study, the safety of the methods used, and obtaining written informed consent from parents. For all patients, liver tests and ultrasounds were performed in a laboratory and a

radiology center in the Tabriz Children's Hospital, respectively.

2-4. Intervention

The intervention included blood sampling and performing trans-abdominal ultrasound.

2-5. Ethical consideration

The present study has been approved by the University Ethics Committee with the code IR.TBZMED.REC.2019.620. Participation in this study began after obtaining permission from patients or their families. The information of all patients was studied and saved in a confidential and coded manner. No additional costs were collected from patients for the examinations. All costs were provided by the project manager and with the support of the Vice Chancellor for Research, Tabriz University of Medical Sciences, Iran.

2-6. Inclusion and exclusion criteria

Inclusion criteria include: 1) patients who were hospitalized during years 2008 to 2009, 2) in Tabriz Children's Hospital, Tabriz University of Medical Sciences, Iran, which is a training, treatment, and referral hospital in the northwest of Iran, 3) patients in the age range of 20-30 years 4) children with hematologic malignancies, 5) children under HD-MTX regimen (doses ≥ 500 mg/m²) for 6 months 6) accessibility to patients or tendency to participate.

Exclusion criteria include: 1) inaccessibility to the patient, 2) incomplete records, 3) death of patient during the last

ten years, 4) dissatisfaction of parents to participate in the study, 5) use of other hepatotoxic drugs, 6) the presence of known liver disease, or chronic hepatitis B or C.

2-7. Data Analyses

Descriptive data were analyzed based on frequency and percentage, and quantitative data were based on mean, standard deviation, median, minimum and maximum using SPSS software version 22.0. The Chi-Square test was used to examine the relationship between variables. P-value < 0.05 was considered statistically significant difference. Improved Kolmogorov-Smirnov test for normality was used.

3- RESULTS

3-1. General Characteristics

In the current study, 30 patients were examined. The demographic characteristics and sonography results of liver, spleen and bile ducts are shown in **Table.1**. In addition, a minimal or mild increase in the echogenicity of the liver parenchyma was observed in 5 children (16.67%) as grade 1 fatty liver, and the mean port vein flow rate increased in 3 cases (10%). The gallbladder, intra-hepatic, and extra-hepatic bile ducts were normal in all patients. There was no evidence of thrombosis in the portal. The hepatofugal and hepatopetal blood flow was normal in all patients. None of the patients had collateral vessels in the paraumbilic and epigastric regions and in the splenic vein.

Table-1: Demographic characteristics and sonography results of liver, spleen and bile ducts in children with HD-MTX regimen.

Variables	Interventional Group	Control Group	Test	P-value
	Number (%) or Mean (SD)	Number (%) or Mean (SD)		
Gender				
Male	20 (46.5%)	23 (53.4%)	X ² =0.453	0.501
Female	20 (54%)	17 (46%)		
Age				
3 years	18 (64.2%)	10 (35.8%)	X ² =4.119	0.249
4 years	9 (37.5%)	15 (62.5%)		
5 years	8 (50%)	8 (50%)		
6 years	5 (41.6%)	7 (58.4%)		
Weight	14.40±1.676	14.02±1.625	t=-1.016	0.313
Time of venipuncture	4.92+1.071	4.70+0.966	t=-0.986	0.327

SD: standard deviation.

3-2. Relationship between liver serum markers and sonography findings in terms of gender and type of malignancy

None of the serum or sonography findings showed a significant difference according to the patients' gender or diagnosis, including between gender and ALT ($P=0.6$), Bill Total ($P=0.6$), Bill Direct ($P=0.3$), Liver span ($P=0.8$), Liver

echogenicity ($P=0.3$), Spleen span ($P=0.8$), and Port vein blood flow velocity ($P=0.3$); and between type of malignancy and ALT ($P=0.2$), Bill Total ($P=0.2$), Bill Direct ($P=0.3$), Liver span ($P=0.8$), Liver echogenicity ($P=0.1$), Spleen span ($P=0.4$), and Port vein blood flow velocity ($P=0.3$), (**Table.2**).

Table 2: Relationship between liver serum markers and liver ultrasound in terms of gender and type of malignancy in children with HD-MTX regimen.

Variable	Mean (standard deviation)	Independent t-test	P- value
Control group	7.95 (1.084)	17.505	0.001
Interventional group	2.65 (1.577)		

3. Relationship between sonography findings

Patients with grade 1 fatty liver had a higher liver size, although this difference

was not significant, including between Liver echogenicity and Liver span ($P=0.06$), Spleen span ($P=0.3$), and Portal Vein Blood Flow ($P=0.4$), (**Table.3**).

Table-3: Relationship between liver and spleen span and Portal Vein Blood Flow with liver echogenicity in children with HD-MTX regimen.

Variable	Control group Number (%)	Interventional group Number (%)	P- value
Low (0-3)	0	31 (77.5%)	0.001
Average (4-7)	11 (30%)	9 (22.5%)	
High (8-10)	29 (70%)	0	

4- DISCUSSION

In this study, 10 years after treating children with hematologic malignancies by HD-MTX regimen, hepatic complications

were evaluated in 30 patients. Based on literature research, there are no comprehensive previous studies on the long-term (i.e. years after treatment) side

effects of HD-MTX in children with malignancy, and most studies have been conducted on adult populations. In the current study, the serum levels of ALT, total bilirubin and direct bilirubin were elevated in 13%, 13%, and 10% of patients, respectively. In terms of the ultrasound findings of gallbladder and intra and extra hepatic bile ducts, liver and spleen span showed abnormally large appearance in 26.6% and 40%, respectively, and the average portal vein flow rate increased in 10% of cases. Liver parenchyma echogenicity increased slightly and homogeneously in 16.6% of cases, demonstrating the evidence of grade I fatty liver. There were no relationships between serum changes or sonography findings and gender or the type of malignancy. In patients with grade 1 fatty liver, liver size showed an increase, but this was statistically insignificant.

In the study of Mashhadi et al., 102 patients with lymphoma and sarcoma and a mean age of 19.5 years, there were no elevations in PT and BILL while AST and ALT increased in 46.9% of patients after HD-MTX treatment. However, the elevated enzymes normalized after 6 months (15). In the study of Perez et al., AST fluctuation was the most common finding after MTX treatment, and there was a significant relationship between MTX dose and increase in AST level. The elevation of AST following MTX injection was transient and returned to normal in all patients within 1-2 weeks (16). Hegyi et al., in their study, reported a significant and dose-dependent hepatotoxicity for MTX after 24 and 48 hours, but there was no relationship between hepatotoxicity and age. On the other hand, liver enzymes decreased after 48 hours (17). Accordingly, short-term elevation in liver enzymes, especially AST and ALT, can be seen after MTX treatment; however, most of these studies have been conducted on adults. In a study by Laharie et al., 44

(8.5%) out of 518 patients showed evidence of severe liver fibrosis on FibroScan or FibroTest, suggesting that severe liver fibrosis can be a rare complication of MTX. This phenomenon was not associated with the net dose of the drug, but it was directly related to other factors such as alcohol abuse and obesity (18). In another study by Lindsay et al., on 54 patients with a mean age of 54 years and a mean duration of MTX treatment of 6.9 years (an average cumulative dose of 4396 mg), 64.8% had normal biopsy findings while premature fat and mild fibrosis were observed in 13% and 20% of the patients, respectively. On the other hand, none of the patients showed advanced fibrosis (19). In these long-term studies, cirrhosis and fibrosis have rarely been reported, which was similar to the results of our study. Moreover, in those developing fibrosis or cirrhosis, the effects of other risk factors, which are more related to adults, have been indicated. In Lindsay et al., there were no significant associations between liver enzymes and pathologic findings in liver biopsy (19).

It should be noted that in the study of Lindsay et al., patients were treated with MTX because of arthritis, not malignancy. In fact, malignancy may be a predisposing factor for severe hepatic complications following MTX therapy. However, in a prospective study by Locasciulli et al., they evaluated 68 children with ALL from the diagnosis to the end of HD-MTX treatment and found no major acute hepatotoxicity for HD-MTX therapy in these children (20). In the study of Kawakatsu S. et al., 320 patients (135 adult and 185 pediatric) treated with HD-MTX were evaluated. They observed a difference in toxicity patterns based on age. Such that HD-MTX was related to higher hazard of nephrotoxicity in adults, and neurotoxicity and hepatotoxicity in children (21). This suggests that a combination of risk factors may influence

the occurrence of severe hepatotoxicity after MTX therapy. In the study of Bath et al., cirrhosis developed in 5% of patients who received long-term low dose MTX (6). Sotoudehmanesh et al., in a retrospective study examined the hepatotoxic effects of MTX in 286 patients with rheumatoid arthritis (RA) over a period of 15 years. Elevated liver enzymes were detected in 23.7% of the patients exposed to MTX for 40.5 ± 34.6 months. The interval between the start of treatment and a rise in liver enzymes was 22.1 ± 22.1 months. The only important factor predicting an increase in liver enzymes was the duration of MTX treatment. The mean duration of treatment was longer in patients with elevated liver enzymes (59.6 ± 42.3 months) than those without increased liver enzymes. There was a statistically significant relationship between the cumulative dose of MTX and elevation of liver enzymes. So, it was concluded that MTX hepatotoxicity was a common complication of long-term MTX therapy, is characterized by a slight increase in liver enzymes and is associated with the duration of treatment (22). In the recent study, contrary to the present one, patients were adults, had a non-malignant disease, and had received low dose MTX over a long period, suggesting that the route of administration may also affect the occurrence of liver complications.

4-1. Study Limitations

The limitation of this study included: 1) a small sample size, 2) patients with malignancy, and 3) the lack of comparison with the control group or adults.

5- CONCLUSION

In this study, we found that long term high-dose MTX therapy could not significantly affect serological parameters of hepatic function (i.e. AST, PT, PTT, total bilirubin, and direct bilirubin) after several years of treatment discontinuance. The main change was seen in the ALT level. None of the observed changes were

related to the type of malignancy or gender. It can also be concluded that treatment with high doses of MTX will not predispose patients to liver cirrhosis and fibrosis in those who have improved and stopped treatment for several years. Nevertheless, some complications include hepatomegaly, splenomegaly, and increased hepatic parenchymal echogenicity, grade I fatty liver, and increased portal vein flow rate. There was no relationship between liver size and the development of grade I fatty liver. Furthermore, we observed no cases of chronic liver involvement in our patients. Based on our results and the findings of other studies, it is recommended to use short-term high-dose MTX and resolve other risk factors to prevent severe liver complications years after the discontinuance of chemotherapy.

In this study, due to the lack of evidence of significant liver involvement, liver biopsy was not indicated for patients. However, because of the possibility of mild complications, as observed here, there is a need to follow up patients for years after therapy discontinuance. Future studies on larger populations of children, especially those with autoimmune diseases, are recommended. It is also suggested to investigate the risk factors and histological features of MTX hepatotoxicity to provide accurate and efficient treatment and follow-up guidelines. The main limitation of this study was the small number of samples and the lack of a control group.

6- ABBREVIATION

DILI: Drug-Induced Liver Injury

MTX: Methotrexate

HDMTX: High-dose Methotrexate

ALL: Acute Lymphoblastic Leukemia

ID: Intermediate-dose

LD: Low-dose

AST: Aspartate Aminotransferase

ALT: Alanine Aminotransferase

PT: Prothrombin time

PTT: Partial thromboplastin time.

7- ACKNOWLEDGMENTS

This study has been supported by the Vice Chancellor for Research, Tabriz University of Medical Sciences, Iran. We would like to thank the staff of Clinical Research Development Unit of Tabriz Children's Hospital, Tabriz University of Medical Sciences and the participants for taking part in the research process.

8- CONFLICT OF INTEREST: None.

9- REFERENCES

- Grijalva J, Vakili K, editors. Neonatal liver physiology. *Seminars in pediatric surgery*; 2013: Elsevier.
- Nishikawa H, Osaki Y. Liver cirrhosis: evaluation, nutritional status, and prognosis. *Mediators of inflammation*. 2015;2015.
- Hamilton LA, Collins-Yoder A, Collins RE. Drug-induced liver injury. *AACN Advanced Critical Care*. 2016;27(4):430-40.
- Gong F, Meng Q, Liu C, Zhao Y. Efficacy and association analysis of high-dose methotrexate in the treatment of children with acute lymphoblastic leukemia. *Oncology letters*. 2019;17(5):4423-28.
- Campbell JM, Bateman E, Stephenson MD, Bowen JM, Keefe DM, Peters MD. Methotrexate-induced toxicity pharmacogenetics: an umbrella review of systematic reviews and meta-analyses. *Cancer chemotherapy and pharmacology*. 2016;78(1):27-39.
- Bath RK, Brar NK, Forouhar FA, Wu GY. A review of methotrexate-associated hepatotoxicity. *Journal of digestive diseases*. 2014;15(10):517-24.
- Feinsilber D, Leoni RJ, Siripala D, Leuck J, Mears KA. Evaluation, Identification, and Management of Acute Methotrexate Toxicity in High-dose Methotrexate Administration in Hematologic Malignancies. *Cureus*. 2018 Jan 8;10(1):e2040. doi: 10.7759/cureus.2040.
- Cheng KF. Association of plasma methotrexate, neutropenia, hepatic dysfunction, nausea/vomiting and oral mucositis in children with cancer. *European journal of cancer care*. 2008;17(3):306-11.
- Hagag AA, Elgamsy MA, El-Asy HM, Mabrouk MM. Protective Role of Silymarin on Hepatic and Renal Toxicity Induced by MTX Based Chemotherapy in Children with Acute Lymphoblastic Leukemia. *Mediterr J Hematol Infect Dis*. 2016 Sep 1;8(1):e2016043. doi: 10.4084/MJHID.2016.043.
- Ahmadzadeh A, Zamani N, Hassanian-Moghaddam H, Hadeiy SK, Parhizgar P. Acute versus chronic methotrexate poisoning; a cross-sectional study. *BMC Pharmacology and Toxicology*2019; 20: 39.
- Traivaree C, Likasitthananon N, Monsereenusorn C, Rujkijyanont P. The effect of intravenous hydration strategy on plasma methotrexate clearance during intravenous high-dose methotrexate administration in pediatric oncology patients. *Cancer Management and Research*. 2018;10:4471.
- Howard SC, McCormick J, Pui C-H, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. *The oncologist*. 2016;21(12):1471.
- Kalb RE, Strober B, Weinstein G, Lebwohl M. Methotrexate and psoriasis: 2009 National Psoriasis Foundation consensus conference. *Journal of the American Academy of Dermatology*. 2009;60(5):824-37.
- Vahedi L, Jabarpour-Bonyadi M, Ghojzadeh M, Hazrati H, Rafeey M. Association Between Outcomes and Demographic Factors in an Azeri Turkish Population With Cystic Fibrosis: A Cross-Sectional Study in Iran From 2001 Through 2014, *Iran Red Crescent Med J*. 2016 ; 18(4):e29615. doi: 10.5812/ircmj.29615.
- Mashhadi M, Mahammadi M, Bakhshipour A, Keikhaei M, Sandoughi M, Heidari Z, et al. High Dose Methotrexat Liver Toxicity. *International Journal of Hematology-Oncology and Stem Cell Research*. 2011:16-9.

16. Perez C, Sutow WW, Wang Y, Herson J. Evaluation of overall toxicity of high-dosage methotrexate regimens. *Medical and Pediatric Oncology*. 1979;6(3):219-28.
17. Hegyi M, Gulácsi A, Cságoly E, Csordás K, Eipel OT, Erdélyi DJ, Müller J, Nemes K, Lautner-Csorba O, Kovács GT. Clinical relations of methotrexate pharmacokinetics in the treatment for pediatric osteosarcoma. *J Cancer Res Clin Oncol*. 2012 Oct;138(10):1697-702. doi: 10.1007/s00432-012-1214-2.
18. Laharie D, Seneschal J, Schaefferbeke T, Doutre M-S, Longy-Boursier M, Pellegrin J-L, et al. Assessment of liver fibrosis with transient elastography and FibroTest in patients treated with methotrexate for chronic inflammatory diseases: a case-control study. *Journal of hepatology*. 2010;53(6):1035-40.
19. Lindsay K, Fraser AD, Layton A, Goodfield M, Gruss H, Gough A. Liver fibrosis in patients with psoriasis and psoriatic arthritis on long-term, high cumulative dose methotrexate therapy. *Rheumatology*. 2009;48(5):569-72.
20. Locasciulli A, Mura R, Fraschini D, Gornati G, Scovena E, Gervasoni A, et al. High-dose methotrexate administration and acute liver damage in children treated for acute lymphoblastic leukemia. A prospective study. *Haematologica*. 1992;77(1):49-53.
21. Kawakatsu S, Nikanjam M, Lin M, Le S, Saunders I, Kuo DJ, Capparelli EV. Population pharmacokinetic analysis of high-dose methotrexate in pediatric and adult oncology patients. *Cancer Chemother Pharmacol*. 2019 Dec;84(6):1339-1348. doi: 10.1007/s00280-019-03966-4. Epub 2019 Oct.
22. Sotoudehmanesh R, Anvari B, Akhlaghi M, Shahraneeni S, Kolahdoozan S. Methotrexate hepatotoxicity in patients with rheumatoid arthritis. *Middle East journal of digestive diseases*. 2010;2(2):104.