Original Article

The Effect of Dextrose Hypotonic vs Saline Hydration on Methotrexate-Induced Nephrotoxicity in Male and Female Rats

Zahra Hasanpour[1,*], Samira Choopani[2,3,*], Farzaneh Ashrafi[1], Ardeshir Talebi[4], Mehdi Nematbaksh[2,5]

[1]Department of Internal Medicine, Oncology-Hematology Section, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Department of Clinical Pathology, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Isfahan[MN] Institute of Basic and Applied Sciences Research, Isfahan, Iran

*These two authors contributed equal work

Abstract

Background: High-dose methotrexate (HDMTX) as a cytotoxic agent might cause various side effects. Hyperhydration has been implemented as the major strategy to decrease the potential risk of toxicities induced by HDMTX. This study aims to assess the renoprotective effect of hydration with dextrose water (DW) 5% versus normal saline (N/S) 0.9% against methotrexate (MTX) induced nephrotoxicity.

Materials and Methods: This experimental animal study has been conducted on 36 Wistar rats (200–250 g) categorized into six groups, including male ($n = 6$) and female ($n = 6$) rats receiving sodium chloride 0.9% saline plus MTX, DW 5% plus MTX, or MTX alone. By the fifth day after the MTX injection, biochemical indexes were measured. The rats were also sacrificed and renal specimens were evaluated microscopically to determine kidney tissue damage (KTD).

Results: The groups were not significantly different with regard to blood urea nitrogen (BUN) ($P = 0.5$), creatinine (Cr) ($P = 0.24$), kidney weight ($P = 0.34$), and urine flow (UF) ($P = 0.5$), while KTD score was remarkably less in the hydrated groups ($P < 0.001$). Weight loss in DW-treated rats was significantly more than N/S-treated ones, and creatinine clearance (CrCl) and urine load (UL) of Cr were statistically similar between males and females in the control group, but significantly lower among the DW5% treated males.

Conclusion: Based on the findings of this study, hydration with N/S was superior to DW5% for the prevention from HDMTX-induced nephrotoxicity. Besides, we found insignificant differences between male versus female rats in response to the hydration for HDMTX-induced renoprotection; however, females probably benefit more.

Keywords: Glucose solution, methotrexate, renal injury, saline solution

Address for correspondence: Prof. Farzaneh Ashrafi, Department of Internal Medicine, Oncology-Hematology Section, Isfahan University of Medical Sciences, Isfahan - 8174673461, Iran.
E-mail: farashrafi@yahoo.com
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Introduction

Methotrexate (MTX) is a well-known cytotoxic agent that has been widely used for the treatment of autoimmune and rheumatologic disorders. This agent has been used in high doses for diverse malignant conditions such as acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma, and osteosarcoma.[1] MTX is a synthetic antimetabolite of folate and the S phase of the cell cycle resulting in the inhibition of DNA synthesis, repair, and cellular replication. This process occurs by irreversible competitive dihydrofolate reductase enzyme inhibition that interferes with the synthetic process of active tetrahydrofolate leading to purine and thymidylic acid synthesis inhibition. This action process finally inhibits DNA synthesis, repair, and cellular replication.[2] Therefore, the tissues with active

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proliferation manner are the most affected ones by this chemotherapeutic agent.[3]

High-dose methotrexate (HDMTX) might cause various side effects including nephrotoxicity, hepatotoxicity, neurotoxicity, mucositis or ulcerative stomatitis, and myelosuppression. MTX is mainly eliminated from the body by the kidneys in two phases of glomerular filtration that occurs passively and by active tubular reabsorption and secretion.[4] Given that, accurate monitoring of drug clearance and precise supportive care for averting potential complications are critical. Therefore, intravenous hydration and leucovorin rescue are two crucial strategies applied for MTX renal toxicity prevention.[5,6]

Hyperhydration has been implemented as the major strategy to decrease the potential risk of toxicities induced by HDMTX by the reduction of MTX precipitation in the renal tubules. Urinary alkalinization has been also utilized by which urine pH increases and the renal clearance of MTX improves. These two strategies have been hypothesized to affect either direct tubular toxicity, pH-dependent precipitation of MTX in the renal tubules, or both.[2] However, the best hydration regimen which can ultimately be the preventive strategy has not been well-elucidated.

The other aspect that has been notified about MTX-induced nephrotoxicity refers to a gender-based renal injury that the male sex is potentially at increased risk compared with females. Nevertheless, there is no information in this regard; however, hypotheses targeted the potential role of estrogen.[7] Herein, it is aimed to compare MTX-induced nephrotoxicity in rats using different hydration regimens, dextrose water (DW) 5% or normal saline (N/S) 0.9%, and gender-based renal complications.

Materials and Methods

Study population

The current experimental animal study has been conducted on 36 Wistar rats (200–250 g), including 18 males and 18 females randomized into six groups, each containing six members. The study has been conducted in the Water and Electrolyte Research Center at Isfahan University of Medical Sciences.

The study protocol was primarily proposed for the Ethics Committee of Isfahan University of Medical Sciences and approved based on code number IR.MUI.MED.REC.1400.007. The study design is compatible with the tenets of the Helsinki declaration version 2013 considering animal rights.

The rats were divided into groups of 18 males and 18 females that each were randomly categorized into three groups, containing 6 members Accordingly, a random number was provided to each subject allocating him/her to one of the intervention groups.

Interventions

The rats were primarily transmitted to the laboratory for adaptation to their environment. Accordingly, the temperature was set to 22–25°C and, they had free access to food and water. Afterward, they were categorized into six groups as follows:

Group 1: Male rats receiving sodium chloride 0.9% saline plus MTX,

Group 2: Female rats receiving sodium chloride 0.9% saline plus MTX,

Group 3: Male rats receiving DW 5% plus MTX,

Group 4: Female rats receiving DW 5% plus MTX,

Group 5: Male rats receiving MTX alone, and

Group 6: Female rats receiving MTX alone.

Groups 1–4 were hydrated with 3 cc of either sodium chloride 0.9% or DW 5% twice a day for three days and groups 5 and 6 did not receive any hydration, while all the groups received 20 mg/kg MTX (Mylan, Switzerland) through intraperitoneal route in the second day of injection.[8]

By the fifth day after the injections, rats had been placed in metabolic cages for 6 h before sacrifice, and a urinary sample was taken from the rats to measure the creatinine clearance (CrCl), the urine flow (UF), and the urine load (UL) of creatinine (Cr). Then, the rats were sacrificed humanely using chloral hydrate (450 mg/kg, Merck, Germany) and xylazine (10 mg/kg, Avacare, China). A blood sample was taken from the rat's myocardial blood flow using a 22 gauge needle to measure blood urea nitrogen (BUN) and Cr.

All the rats were weighed at the study baseline and before being sacrificed to measure the impact of methotrexate therapy on their body weight. The measurements were done using a standard weight with a diagnostic accuracy of 0.0001 gr.

Afterward, the kidneys were extracted, weighed, and put into formalin 10% (Behrad Shimi, Iran). After tissue processing, the kidney tissue was fixed in paraffin (Exin paraffin, Iran) for a period of 2–3 h and then, biopsied in cuts with 5-µ-thick sections. At least, ten sections were obtained from each kidney. The specimens were dyed using hematoxylin and eosin (H and E) staining and studied under *10 and *40 microscopes by a skilled pathologist blinded to the intervention groups. The kidney tissue damage (KTD) score was scored in 1–4 categories according to the severity of the acute tubular injury [Table 1].

Statistical analysis

The obtained data were entered into the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 23. Categorical data were presented in absolute numbers and percentages, while continuous ones were reported as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) was applied to compare the data between the groups, and the Student *t*-test was used to compare the data between the sexes. The Kruskal–Wallis and Mann–Whitney U tests were employed to compare the severity of kidney injury

between the groups. A *P* value of less than 0.05 was assigned as significant.

Results

Evaluation of hydration methods regardless of gender difference

The groups were not significantly different with regard to BUN ($P = 0.5$), Cr (P- =0.24), kidney weight ($P = 0.34$), and UF ($P = 0.5$), while the KTD score was remarkably different between the groups ($P < 0.001$). Further assessments revealed significantly lower scores of KTD by the comparison of both DW5%-treated and N/S-treated rats with the controls ($P < 0.05$). Besides, the control group lost weight significantly more than N/S-treated ones, while those who were hydrated with DW5% presented statistically more significant weight loss than the controls ($P < 0.05$). The other finding refers to significantly lower levels of CrCl and UL of creatinine among DW5%-treated rats compared with the controls ($P < 0.05$). Detailed information is presented in Table 2.

As shown in Table 2, hydration regardless of its regimen was accompanied by less KTD than the controls (P value < 0.001).

Evaluation of hydration method considering gender difference

Gender assessment of KTD in the control group revealed insignificant differences between males and females ($P = 0.09$). The comparison of male KTD in the groups revealed significantly more severe KTD in the control males than in the intervention groups ($P < 0.05$). Similar patterns were observed for females ($P < 0.05$), as well [Figure 1].

BUN ($P = 0.001$) and Cr ($P = 0.05$) levels in the females of the control group were higher than in the males. Despite the normal range of BUN and creatinine in all groups [Table 2], creatinine levels were significantly higher in the control than in the DW5%-treated females. BUN assessments represented statistically higher levels in the N/S-treated males than the controls [Figure 2a and b]. CrCl and UL of Cr were statistically similar between males and females in the control group, but significantly lower among the males treated with DW5% than the controls [Figure 2c and d]. Besides, there was no gender-based or intervention-based difference in the UF assessments [Figure 2e]. Weight loss was remarkably more notable in the females than the males treated with DW5% ($P = 0.03$).

In addition, both males and females treated with DW5% lost statistically more weight than those treated with N/S. This significant difference was noted for DW5%-treated females in comparison to control females, as well ($P < 0.05$) [Figure 2f].

Table 1: Scoring system to classify renal histologic injury: Grade of histologic changes as glomerulopathy, tubulopathy, epithelial lining distortion, and edema and enlargement in capillaries[9]

Score	Percent of pathologic changes
1: Mild	Up to 25%
2: Moderate	25–50%
3: Severe	50–75%
4: Very severe	Over 75%

Discussion

The current study has been conducted to assess the most appropriate hyperhydration regimen to minimize the potentiality of methotrexate-induced nephrotoxicity. Accordingly, we have assessed the following three facets: 1) the renoprotective effect of hydration, 2) the best hydration strategy to the best of our knowledge, the current study is the first one assessing the application of different hydration regimens to prevent the potential nephrotoxicity induced by methotrexate treatment, and 3) the gender-based differences regarding methotrexate-induced nephrotoxicity and response to the hydration.

Accordingly, we found that the hydration of the rats regardless of the administered fluid led to dramatic less

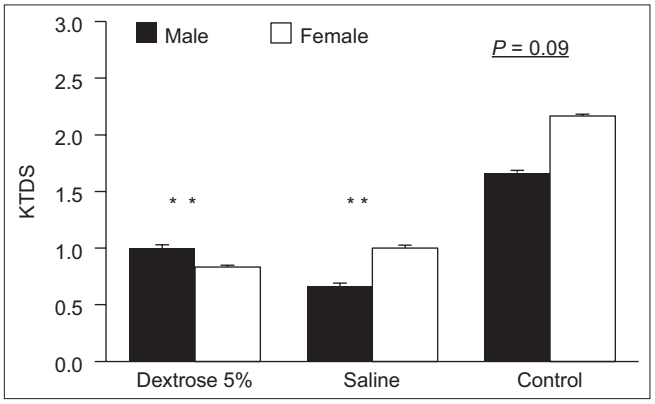

Figure 1: Gender-based assessment of KTD score (KTDS). *Statistically significant difference with the control group

Table 2: The effect of different interventions on the assessed parameters

Group	BUN (mg/dl)	Cr (mg/dl)	KW (g/100 g BW)	BW (% change)	CrCl (μl/min)	UF (μl/min)	UL of Cr (μg/min)	KTDS
Dextrose water 5%	20.8±1.8	0.45±0.02	0.72±0.02	−10.1±−1.5*	307.8±36.9*	5.5±0.9	1.4±0.2*	0.92±0.1*
Sodium chloride 0.9%	22.2±0.9	0.50±0.02	0.69±0.02	−4.27±−1.4*	366.1±39.8	4.7±0.6	1.8±0.2	0.83±0.2*
Control group	20.1±0.9	0.49±0.03	0.70±0.01	-6.45±1.6	470.5±65.0	4.3±0.5	2.2±0.3	1.92±0.1
P	0.5	0.24	0.34	0.03	0.07	0.5	0.06	<0.001

*Statistically significant difference with the control group

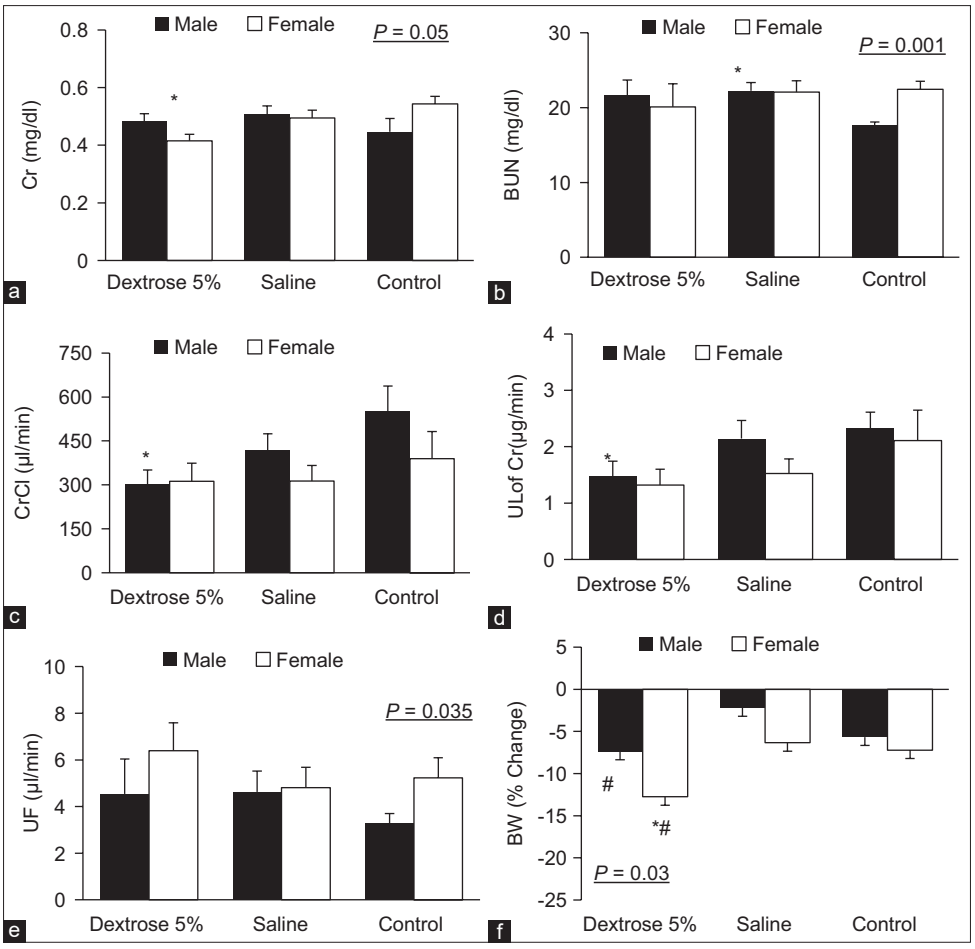

Figure 2: Gender-based assessment of the study parameters (a) Creatinine (b) BUN (c) creatinine clearance (d) urine load of creatinine (e) urine flow, and (f) body weight loss. *Statistically significant difference with the control group. #Statistically significant difference with the intervention group. The absolute represented *P* values show the difference between males and females in that group

KTD than the controls who received methotrexate only; however, kidney function indexes as BUN, Cr, CrCl, UF, and UL of Cr changes were not as clear as pathologic changes among the groups. These findings are consistent with the previous studies regarding the necessity of hydration before high-dose administration of methotrexate to minimize the potential risk of nephrotoxicity.[2,10,11] The trials performed on human cases emphasized that it is essential to start aggressive hydration of the patients within 12 h before methotrexate treatment and continue it for 24–48 h.[6]

KTD scores were remarkedly worse among the controls compared to the hydrated rats regardless of the applied regimen [Figure 3]. The point that should be notified is the significance of KTD in advance to the clinical manifestations of renal injury, as creatinine and BUN were within the normal limits in all the groups, while the KTD scores were initiated in all groups with the most significant progress in the non-hydrated controls. However, there was no difference between those treated with N/S versus DW5%. This fact emphasizes that renal injury might initiate at very early stages of methotrexate use, but be underestimated.

Acute kidney injury induced by methotrexate occurs in 2–50% of the cases treated with HDMTX regarding the underlying risk factors and the definitions.[12] Tubular injury due to precipitation of MTX or one of its metabolites in the tubular lumen (intratubular obstruction) and the reactive inflammatory processes following tubular precipitation[13] or because of the afferent capillary constriction that leads to mesangial or tubular cells death is the most common pathology noticed by methotrexate-induced nephrotoxicity[14]; however, it is typically reversible.[15]

Hayashi and colleagues presented a gradual dose-dependent decrease in the estimated glomerular filtration rate assessing kidney function during a year.[16] The incidence of methotrexate-induced nephrotoxicity has been well-documented in high-dose treatments; however, it was a rare condition in low-dose approaches. Recent long-term assessments also revealed renal injury by low-dose treatments, which refers to the gradual progress in renal function.[6,17,18] However, not precisely, we want to emphasize the gradual tubular changes in the kidneys before the clinical manifestations of kidney dysfunction that confirm our finding.

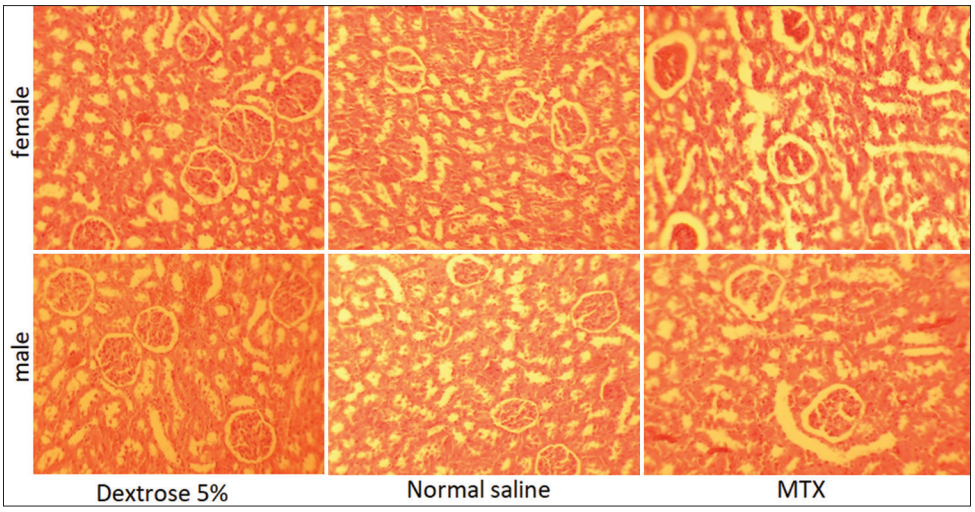

Figure 3: The sample image of kidney tissue stained with H and E to examine tissue damage in the kidney of six experimental groups. Higher damage scores were observed in the groups treated only with MTX without hydration (control groups)

Although, the main scope of this study was to highlight the hydration strategy by which the ultimate renoprotection occurs, however, we have not achieved considerable outcomes on the basis of biochemical elements, in this regard that might not be generalizable due to the sample population, but significant weight loss occurrence among the rats treated with DW5% in comparison to better weight-perseverance in N/S-treated rats than the controls, has convinced us to prefer N/S rather than DW5%. Lower levels of CrCl and UL of Cr are the other factors leaning the attention toward N/S.

Weight loss due to chemotherapy with HDMTX is a common condition reported in the literature.[19] One of the main secondary toxic side effects of antimitotic agents is mucositis, at the same time food ingestion is decreased not only because of painful mucosal lesions but also because central regulation of food intake can contribute to the anorexic effect. MTX induces a marked decrease in food intake in rats. It is demonstrated that weight loss begins in the first few days of MTX administration.[20,21] We found no hypothesis for reasoning the mechanism by which N/S use led to less weight loss in comparison to the control group or significantly higher weight loss in DW5%-treated rats. Hyperglycemia can affect the pharmacokinetics and pharmacodynamics of drugs.[22] It is demonstrated that plasma concentration of MTX is significantly higher in diabetic rats after administration of intravenous MTX than in control rats. This resulted in a significantly increased mean residence time in diabetic rats.[23] It should be investigated if the toxic effects of MTX were higher in dextrose-treated rats. And if the weight loss is related to increased toxic effects.

However, our leaning toward N/S versus DW5% has been previously notified. Mangum *et al*. presented better methotrexate clearance among the cases receiving higher sodium load assessing methotrexate clearance 24 h after the treatment.[24] The other study assessing this issue by Kinoshita *et al*. previously reported that hydration with a higher sodium dose facilitated methotrexate clearance after HDMTX.[25] The mechanisms by which the serum sodium concentration affects MTX clearance include its effect on renal glomerular filtration and/or active secretion of methotrexate via the proximal tubules.[26] It has been postulated that sodium, as a key regulator of tubuloglomerular feedback, can transiently increase the glomerular filtration rate (GFR). The increased GFR then results in increased renal clearance of MTX.[25]

Generally, the risk of nephrotoxicity was higher among females than males according to the manifestations of renal function, including BUN, creatinine, CrCl, UF, and UL of Cr, even if the differences were not statistically significant or the measurements were within the normal limits. Nevertheless, we found that hydration leads to the disappearance of the differences in kidney function indices; a condition that shows more benefit for females than males from hydration.

Most of the studies in the literature opposed us as the male gender is potentially a risk factor for HDMTX-induced nephrotoxicity.[4,7] We assume that small a sample population might be the reason for our inverse outcomes.

Limitations

The small sample population and the short period of urine accumulation limited to 6 h rather than 18 h are the most significant limitations of this study. Further studies with larger sample populations and assessing different hydration fluids such as half saline are strongly recommended.

Conclusion

Based on the findings of this study, hydration with N/S was superior to DW5% for the prevention of HDMX-induced nephrotoxicity. Besides, we found insignificant differences between male versus female rats in response to the hydration for HDMTX-induced renoprotection; however, females probably benefit more.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. 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 Manag Res 2018;10:4471-8.
2. Chen AR, Wang YM, Lin M, Kuo DJ. High-dose methotrexate in pediatric acute lymphoblastic leukemia: Predictors of delayed clearance and the effect of increased hydration rate on methotrexate clearance. Cureus 2020;12:e8674.
3. Khan ZA, Tripathi R, Mishra B. Methotrexate: A detailed review on drug delivery and clinical aspects. Expert Opin Drug Deliv 2012;9:151-69.
4. Amitai I, Rozovski U, El-Saleh R, Shimony S, Shepshelovich D, Rozen-Zvi B, *et al*. Risk factors for high-dose methotrexate associated acute kidney injury in patients with hematological malignancies. Hematol Oncol 2020;38:584-8.
5. Svahn T, Mellgren K, Harila-Saari A, Åsberg A, Kanerva J, Jónsson Ó, *et al*. Delayed elimination of high-dose methotrexate and use of carboxypeptidase G2 in pediatric patients during treatment for acute lymphoblastic leukemia. Pediatr Blood Cancer 2017;64.
6. Howard SC, McCormick J, Pui CH, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. Oncologist 2016;21:1471-82.
7. Wiczer T, Dotson E, Tuten A, Phillips G, Maddocks K. Evaluation of incidence and risk factors for high-dose methotrexate-induced nephrotoxicity. J Oncol Pharm Pract 2016;22:430-6.
8. Abdel-Raheem IT, Khedr NF. Renoprotective effects of montelukast, a cysteinyl leukotriene receptor antagonist, against methotrexate-induced kidney damage in rats. Naunyn Schmiedebergs Arch Pharmacol 2014;387:341-53.
9. Aldemir M, Okulu E, Kösemehmetoğlu K, Ener K, Topal F, Evirgen O, *et al*. Evaluation of the protective effect of quercetin against cisplatin-induced renal and testis tissue damage and sperm parameters in rats. Andrologia 2014;46:1089-97.
10. Karremann M, Sauerbier J, Meier C, Vetter C, Schneider H, Buchholz B, *et al*. The impact of prehydration on the clearance and toxicity of high-dose methotrexate for pediatric patients. Leuk Lymphoma 2014;55:2874-8.
11. Mikkelsen TS, Mamoudou AD, Tuckuviene R, Wehner PS, Schroeder H. Extended duration of prehydration does not prevent nephrotoxicity or delayed drug elimination in high-dose methotrexate infusions: A prospectively randomized cross-over study. Pediatr Blood Cancer 2014;61:297-301.
12. Perazella MA, Rosner MH. Drug-induced acute kidney injury. Clin J Am Soc Nephrol 2022;17:1220-33.
13. Garneau AP, Riopel J, Isenring P. Acute methotrexate-induced crystal nephropathy. N Engl J Med 2015;373:2691-3.
14. Kitamura M, Kitamura S, Fujioka M, Kamijo R, Sato S, Sawayama Y, *et al*. Methotrexate-induced acute kidney injury in patients with hematological malignancies: Three case reports with literature review. Renal Replacement Therapy 2018;4:1-8.
15. Medrano C, Oberic L, Puisset F, Recher C, Larrieu-Ciron D, Ysebaert L, *et al*. Life-threatening complications after high-dose methotrexate and the benefits of glucarpidase as salvage therapy: A cohort study of 468 patients. Leuk Lymphoma 2021;62:846-53.
16. Hayashi K, Sada K-E, Asano Y, Asano SH, Yamamura Y, Ohashi K, *et al*. Risk of higher dose methotrexate for renal impairment in patients with rheumatoid arthritis. Sci Rep 2020;10:18715.
17. Karstila K, Korpela M, Sihvonen S, Mustonen J. Prognosis of clinical renal disease and incidence of new renal findings in patients with rheumatoid arthritis: Follow-up of a population-based study. Clin Rheumatol 2007;26:2089-95.
18. Amin A, Effat D, Goher N, Ramadan B. Tc-99m diethylenetriamine pentaacetic acid (DTPA): Is it reliable for assessment of methotrexate-induced cumulative effect on renal filtration in rheumatoid arthritis patients? Rheumatol Int 2013;33:3059-63.
19. François M, Takagi K, Legrand R, Lucas N, Beutheu S, Bôle-Feysot C, *et al*. Increased ghrelin but low ghrelin-reactive immunoglobulins in a rat model of methotrexate chemotherapy-induced anorexia. Front Nutr 2016;3:23.
20. Boukhettala N, Leblond J, Claeyssens S, Faure M, Le Pessot F, Bôle-Feysot C, *et al*. Methotrexate induces intestinal mucositis and alters gut protein metabolism independently of reduced food intake. Am J Physiol Endocrinol Metab 2009;296:E182-90.
21. Myers CE, Hoelzinger DB, Truong TN, Chew LA, Myles A, Chaudhuri L, *et al*., Chemotherapy can induce weight normalization of morbidly obese mice despite undiminished ingestion of high fat diet. Oncotarget 2017;8:5426-38.
22. Mashayekhi-Sardoo H, Mohammadpour AH, Nomani H, Sahebkar A. The effect of diabetes mellitus on pharmacokinetics, pharmacodynamics and adverse drug reactions of anticancer drugs. J Cell Physiol 2019;234:19339-51.
23. Park JM, Moon CH, Lee MG. Pharmacokinetic changes of methotrexate after intravenous administration to streptozotocin-induced diabetes mellitus rats. Res Commun Mol Pathol Pharmacol 1996;93:343-52.
24. Mangum R, Bernhardt MB, Cheng WS, Schafer ES, Berg SL, Foster JH. Do intravenous fluid substitutions influence methotrexate clearance? An unanticipated impact of an intravenous sodium bicarbonate drug shortage. Pediatr Blood Cancer 2020;67:e28334.
25. Kinoshita A, Kurosawa Y, Kondoh K, Suzuki T, Manabe A, Inukai T, *et al*. Effects of sodium in hydration solution on plasma methotrexate concentrations following high-dose methotrexate in children with acute lymphoblastic leukemia. Cancer Chemother Pharmacol 2003;51:256-60.
26. Mikkelsen TS, Thorn CF, Yang JJ, Ulrich CM, French D, Zaza G, *et al*. PharmGKB summary: Methotrexate pathway. Pharmacogenet Genomics 2011;21:679-86.