

# Delayed High-dose Methotrexate Excretion and Influencing Factors in Osteosarcoma Patients

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## Abstract

**Background:** High-dose methotrexate (HD-MTX) with folinic acid (leucovorin) rescue is “gold standard” therapy for osteosarcoma. Plasma concentrations of methotrexate (MTX) are closely related to its efficacy and toxicity. Delayed excretion of MTX can lead to serious adverse reactions that may result in treatment cessation, irreversible organ damage, and death. This study focused on the incidence of delayed excretion of MTX in Chinese osteosarcoma patients.

**Methods:** A total of 1277 osteosarcoma patients were treated with HD-MTX chemotherapy (4291 cycles) from 2010 to 2015. Factors that could influence delayed excretion of MTX (gender, age, number of chemotherapy cycles, and serum concentration of MTX) were analyzed.

**Results:** The incidence of delayed excretion of MTX (serum concentrations at 24 h [ $C_{24h}$ ]  $>5 \mu\text{mol/L}$ ) and severe delayed excretion of MTX ( $C_{24h} >20 \mu\text{mol/L}$ ) were 6.19% and 0.86% per patient, and 2.31% and 0.26% per cycle of treatment, respectively. The incidence of severe delayed excretion of MTX was associated with gender, age, and  $C_{24h}$ .

**Conclusions:** Precaution of delayed excretion of MTX is needed during osteosarcoma treatment using HD-MTX. An optimal individualized rescue strategy can be created with consideration of gender, age, and  $C_{24h}$ .

**Key words:** Delayed Excretion; Methotrexate; Osteosarcoma; Therapeutic Drug Monitoring

## INTRODUCTION

High-dose methotrexate (HD-MTX) with folinic acid (leucovorin) rescue is an important chemotherapy regimen used commonly for osteosarcoma.<sup>[1]</sup> The study has demonstrated a 5-year survival in osteosarcoma patients treated with neoadjuvant therapy of 65–75%, and HD-MTX (8–12 g/m<sup>2</sup>) has been shown to be the most effective single agent with a response rate of approximately 30%.<sup>[2]</sup> Hydration, alkalization, and leucovorin rescue have evolved to routine clinical administration since Jaffe started to use HD-MTX to treat osteosarcoma in 1972,<sup>[3]</sup> but delayed excretion of methotrexate (MTX) can occur to cause gastrointestinal reactions, oral mucositis, increases in levels of liver enzymes, neurotoxicity, and hematologic toxicity.<sup>[4-6]</sup> These serious adverse effects can lead to treatment cessation, irreversible damage to organs, and death.

A few studies have focused on the incidence of delayed excretion of MTX in Chinese populations and the factors that influence such delayed excretion. We analyzed serum concentrations of MTX in 1277 patients treated with HD-MTX chemotherapy (4291 cycles) in Beijing Jishuitan Hospital (Beijing, China).

The present study was aimed to (i) ascertain the incidence of delayed excretion of MTX among osteosarcoma patients treated with HD-MTX; (ii) evaluate the correlation between

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the gender, age, number of chemotherapy cycles, and serum concentrations of MTX at different times and delayed excretion of MTX; (iii) provide a basis for risk prevention of delayed excretion of MTX; (iv) provide a basis for an individualized rescue strategy.

## METHODS

### Subjects

Serum concentrations of MTX for osteosarcoma patients monitored at our hospital from January 2010 to December 2015 were acquired via the Viva-E® system (Siemens Healthcare, Forchheim, Germany) and were retrospectively analyzed.

Inclusion criteria were patients (i) with a clinical presentation of osteosarcoma and histopathologic confirmation of osteosarcoma by a biopsy specimen; (ii) Grade 1 or better recovery from adverse events of previous treatment according to the US National Cancer Institute – Common Terminology Criteria for Adverse Events (v4.0); (iii) Eastern Cooperative Oncology Group performance status of 0 or 1.

The study protocol was approved by the Ethics Committee of Beijing Jishuitan Hospital. Moreover, all patients provided written informed consent.

### Chemotherapy and rescue strategy

The neoadjuvant chemotherapy plan used to treat osteosarcoma in our department is preoperative chemotherapy, surgery, and postoperative chemotherapy. Commonly used chemotherapy drugs are HD-MTX, ifosfamide, cisplatin, and adriamycin. Hydration and alkalization are implemented 12 h before MTX infusion using 200 ml of 5% sodium bicarbonate ( $\text{NaHCO}_3$ ), 10 ml of 15% potassium chloride, as well as 1 L of 5% glucose and 5% glucose and normal saline (GNS). At the end of the first stage of hydration and alkalization, 5% glucose and 5% GNS (1 L) + 5%  $\text{NaHCO}_3$  (100 ml) + vincristine (2 mg) is administered. The next day, MTX (10 g/m<sup>2</sup>) is added to 5% glucose (500 ml) and infused for 4–6 h. After completion of MTX infusion for 6–8 h, leucovorin rescue (12 mg, q6h) is started until the serum concentration of MTX falls to 0.05  $\mu\text{mol/L}$ . If delayed excretion occurs, the leucovorin dose is increased. During chemotherapy, the patient is given  $\text{NaHCO}_3$  tablets (1.0 g, p.o., tid) and allopurinol (200 mg, p.o., tid). Then, 24-h urine output is recorded, and urine pH controlled to lie within the range of 7–9.

### Monitoring of the serum concentration of methotrexate

After MTX infusion for 0, 24, 48, and 72 h, contralateral venous blood (2 ml) was taken. If the serum concentration of MTX at 72 h ( $C_{72\text{h}}$ ) was  $>0.05 \mu\text{mol/L}$ , an additional 2 ml of contralateral venous blood was obtained every 24 h until the serum concentration of MTX decreased to  $<0.05 \mu\text{mol/L}$ .

### Patient group

Patients were grouped by age as “children” ( $\leq 14$  years) and “adults” ( $>14$  years), as well as by the number of chemotherapy cycles received (1–3 and 4–11 cycles).

Delayed excretion of MTX was defined as  $C_{24\text{h}} > 5 \mu\text{mol/L}$  after MTX infusion, in accordance with the literatures.<sup>[7,8]</sup>  $C_{24\text{h}}$  of 5–20  $\mu\text{mol/L}$  was classified as “mild–moderate” delayed excretion, and  $C_{24\text{h}} > 20 \mu\text{mol/L}$  as “severe” delayed excretion.

### Statistical analysis

Data were analyzed using SPSS version 20.0 (IBM, Armonk, NY, USA). Data was presented as mean  $\pm$  standard deviation (SD), median ( $P_{25}$ ,  $P_{75}$ ), or percentage. The influence of osteosarcoma patients’ gender, age, and number of chemotherapy cycles on delayed excretion of MTX was evaluated by Chi-square test, and the correlation between serum concentration of MTX and delayed excretion of MTX was evaluated by the Mann-Whitney  $U$  test at  $\alpha = 0.05$ .

## RESULTS

### Patients

A total of 1277 patients (mean age,  $18.5 \pm 10.1$  years) formed the study cohort. Among these patients, there were 512 children (40.09%) and 765 adults (59.91%). In addition, 821 patients (64.29%) were male and 456 (35.71%) were female. A total of 4291 cycles of chemotherapy were administered for the 1277 patients; 681 patients received 1–3 cycles each and 596 patients received 4–11 cycles each. Mean serum concentrations of MTX are shown in Table 1.

### Delayed excretion of methotrexate

Of the 1277 patients, delayed excretion of MTX ( $C_{24\text{h}} > 5 \mu\text{mol/L}$ ) was observed in 79 patients (6.19%), of which 68 (5.32%) had mild–moderate delayed excretion and 11 (0.86%) had severe delayed excretion. Of the 4291 cycles of chemotherapy, delayed excretion of MTX occurred in 99 cycles (2.31%), of which 88 (2.05%) were mild–moderate delayed excretion and 11 (0.26%) were severe delayed excretion.

### Influence of gender on delayed excretion of methotrexate

Of the 79 patients (6.19%) with delayed excretion of MTX, 43 (5.24%) were male and 36 (7.89%) were female [Table 2]. The incidence of delayed excretion of MTX between males and females showed no significant difference ( $P = 0.059$ ). For the 11 patients with severe delayed excretion of MTX, the incidence of delayed excretion of MTX showed a significant difference between males and females (0.37% vs. 1.75%,  $P = 0.024$ ).

**Table 1: Mean concentration ( $\mu\text{mol/L}$ ) of MTX in serum ( $n = 1277$ )**

Time	Value
0 h	1105.65 $\pm$ 308.71
24 h	1.66 $\pm$ 4.82
48 h	0.12 $\pm$ 0.58
72 h	0.05 $\pm$ 0.09

Data are shown as mean  $\pm$  standard deviation. MTX: Methotrexate.

### Influence of age on delayed excretion of methotrexate

Of the 79 patients (6.19%) with delayed excretion of MTX, 27 (5.27%) were children and 52 (6.80%) were adults [Table 2]. The incidence of delayed excretion of MTX showed no significant difference between different age groups ( $P = 0.268$ ). For the 11 patients with severe delayed excretion of MTX, the incidence of delayed excretion showed a significant difference between children and adults (1.56% vs. 0.39%,  $P < 0.001$ ).

### Influence of the number of chemotherapy cycles on delayed excretion of methotrexate

For the 99 chemotherapy cycles that resulted in delayed excretion of MTX, the incidence of delayed excretion of MTX showed no significant difference between 1–3 and 4–11 cycles (2.32% vs. 2.28%,  $P = 0.571$ ) [Table 3]. For the 11 cycles that resulted in severe delayed excretion of MTX, there was no significant difference between 1–3 and 4–11 cycles ( $P = 0.616$ ).

### Serum concentrations of methotrexate of patients with severe delayed excretion of methotrexate

Of the 11 patients with severe delayed excretion of MTX, 3 were male, 8 were female; 8 were children and 3 were adults [Table 4]. They received between 1 and 5 cycles. Specifically, nine patients received 1–3 cycles and two patients received 4–5 cycles. Serum concentration of MTX  $<0.05 \mu\text{mol/L}$  was defined as the standard of excretion. The mean time of the 11 patients to reach this standard was  $12.36 \pm 6.15$  days (shortest was 7 days; longest time was 28 days). With sufficient leucovorin rescue, none of the 11 patients experienced a potentially pathogenic complication and did not require invasive treatment, and all proceeded to the next cycle of chemotherapy.

### Correlation between serum concentration of methotrexate and delayed excretion of methotrexate

Patients with delayed excretion of MTX had a  $C_{0h}$  that was significantly higher than that of patients with normal excretion (1310.00 [1122.12, 1490.00] vs. 1100.00 [940.82, 1260.00]  $\mu\text{mol/L}$ ,  $P < 0.001$ ) [Table 5].  $C_{0h}$  showed no significant difference between patients with mild–moderate delayed excretion of MTX and those with severe delayed excretion of MTX [Table 6]. Patients with delayed excretion of MTX had a  $C_{24h}$  that was significantly higher than that of patients with normal excretion (6.84 [5.76, 9.00] vs. 1.20 [0.86, 1.61]  $\mu\text{mol/L}$ ,  $P < 0.001$ ) [Table 5]. Patients with severe delayed excretion of MTX had a  $C_{24h}$  that was significantly higher than that of patients with mild–moderate delayed excretion of MTX (52.56 [23.40, 101.40] vs. 6.70 [5.79, 8.36]  $\mu\text{mol/L}$ ,  $P < 0.001$ .) [Table 6].

## DISCUSSION

We assessed retrospectively the HD-MTX chemotherapy that 1277 osteosarcoma patients received in the past 6 years in our department. The incidences of delayed excretion ( $C_{24h} > 5 \mu\text{mol/L}$ ) and severe delayed excretion

( $C_{24h} > 20 \mu\text{mol/L}$ ) of MTX among these 1277 patients after administration of HD-MTX chemotherapy were 6.19% and 0.86%, and 2.31% and 0.26% of the 4291 chemotherapy cycles in total, respectively. These data are in accordance with the results of a retrospective study of 790 osteosarcoma patients by Bacci *et al.*,<sup>[9]</sup> who also classified  $C_{24h} > 5 \mu\text{mol/L}$  and  $C_{24h} > 20 \mu\text{mol/L}$  as delayed excretion and severe delayed excretion of MTX, respectively. The incidences of delayed excretion and severe delayed excretion of MTX reported in their study were 8.7% and 0.4%, respectively.

### Influence of gender on delayed excretion of methotrexate

Evaluation of the 11 patients with severe delayed excretion of MTX revealed that severe delayed excretion of MTX was associated with gender. The incidence of severe delayed excretion of MTX in female patients (1.75%) was significantly higher than that in male patients (0.37%). For the 79 patients with delayed excretion of MTX, no significant difference was observed between males and females with regard to the incidence of delayed excretion of MTX. This finding may be related (at least in part) to the male:female ratio (821:456) of the 1277 patients in our study. Few studies on patients treated with HD-MTX have reported the influence of gender on pharmacokinetic parameters or delayed excretion of MTX. Several studies have explored the influence of gender on the efficacy/toxicity of HD-MTX

**Table 2: Incidence of delayed excretion of MTX according to gender and age**

Items	Total number of Patients ( $N = 1277$ )	Patients with delayed MTX excretion ( $n = 79$ ), $n$ (%)	Patients with severe delayed MTX excretion ( $n = 11$ ), $n$ (%)
Gender			
Male	821	43 (5.24)	3 (0.37)
Female	456	36 (7.89)	8 (1.75)
$\chi^2$	–	3.57	5.10
$P$	–	0.059	0.024
Age (years)			
$\leq 14$	512	27 (5.27)	8 (1.56)
$> 14$	765	52 (6.80)	3 (0.39)
$\chi^2$	–	1.23	1231.62
$P$	–	0.268	$< 0.001$

–: Not applicable; MTX: Methotrexate.

**Table 3: Incidence of delayed excretion of MTX according to the number of cycles of chemotherapy**

Cycles of chemotherapy	Total cycles ( $N = 4291$ ), $n$	Cycles with delayed MTX excretion ( $n = 99$ ), $n$ (%)	Cycles with severe delayed MTX excretion ( $n = 11$ ), $n$ (%)
1–3	3020	70 (2.32)	9 (0.30)
4–11	1271	29 (2.28)	2 (0.16)
$\chi^2$	–	0.32	0.25
$P$	–	0.571	0.616

–: Not applicable; MTX: Methotrexate.

**Table 4: Serum concentrations of MTX of 11 patients with severe delayed excretion of MTX**

Patient number	Gender	Age (years)	Cycles of chemotherapy	Serum MTX concentration (μmol/L)						Days to reach the standard of excretion
				0 h	24 h	48 h	72 h	96 h	120 h	
1	Female	5	3	690	23.40	1.56	0.69	0.40	0.32	8
2	Female	8	3	1060	46.00	40.00	0.10	0.55	0.24	10
3	Female	9	2	670	52.92	6.60	1.49	0.76	0.45	10
4	Female	9	1	1530	50.76	4.20	1.08	0.72	0.31	10
5	Male	11	1	970	21.60	1.50	0.22	0.10	0.09	7
6	Female	12	1	1130	52.56	5.04	1.17	0.78	0.48	9
7	Male	13	4	1100	20.52	1.45	0.63	0.25	0.11	9
8	Female	14	5	1040	239.76	10.80	7.20	6.70	3.24	16
9	Female	18	2	1810	57.96	3.66	0.77	0.32	0.17	11
10	Female	21	2	1940	112.32	14.04	5.20	2.78	1.36	28
11	Male	49	2	1030	101.40	30.40	6.54	3.72	6.54	18

MTX: Methotrexate.

**Table 5: Serum concentration of MTX (μmol/L) in patients with normal excretion and delayed excretion of MTX**

Time	Normal excretion (n = 1198)	Delayed excretion (n = 79)	U	P
0 h	1100.00 (940.82, 1260.00)	1310.00 (1122.12, 1490.00)	-7.56	<0.001
24 h	1.20 (0.86, 1.61)	6.84 (5.76, 9.00)	-17.03	<0.001

Data are shown as median (P<sub>25</sub>, P<sub>75</sub>). MTX: Methotrexate.**Table 6: Serum concentration of MTX (μmol/L) in patients with mild-moderate and severe delayed excretion**

Time	Mild-moderate delayed excretion (n = 68)	Severe delayed excretion (n = 11)	U	P
0 h	1315.00 (1145.00, 1454.97)	1060.00 (970.00, 1530.00)	-1.82	0.069
24 h	6.70 (5.79, 8.36)	52.56 (23.40, 101.40)	-5.30	<0.001

Data are shown as median (P<sub>25</sub>, P<sub>75</sub>). MTX: Methotrexate.

chemotherapy. For instance, Holmboe *et al.*<sup>[10]</sup> evaluated retrospectively 288 cycles of the HD-MTX chemotherapy received by 65 osteosarcoma patients. The female:male ratio with regard to the maximum concentration of alanine transaminase was 1.7:1.0. Gender was one of the factors influencing liver toxicity but did not influence renal toxicity or delayed excretion of MTX.

### Influence of age on delayed excretion of methotrexate

We also found an association between age and severe delayed excretion of MTX. The incidence of severe delayed excretion of MTX in children (1.56%) was significantly higher than that in adults (0.39%). However, of the 79 patients with delayed excretion of MTX, the incidence of delayed excretion of MTX in adults (6.8%) was higher than that in children (5.27%). This difference may be explained by the small number of patients (11) with severe delayed excretion of MTX. Wang *et al.*<sup>[11]</sup> studied pharmacokinetics in osteosarcoma patients of different age groups (≤10, 11–14, 15–17, and ≥18 years) and identified the influence of age on the MTX concentration in serum. Using an identical dose of MTX, they found that the younger the age of patients, the lower was the serum concentration of MTX they had 6 and 24 h after MTX administration and, therefore, the lower the risk of delayed excretion.

### Influence of the number of chemotherapy cycles on delayed excretion of methotrexate

In the study by Bacci *et al.*,<sup>[9]</sup> the number of cycles of HD-MTX chemotherapy received by 790 patients was 1–10. They reported that the risk of delayed excretion of MTX during the first three cycles was significantly greater than that during subsequent cycles, and that severe delayed excretion of MTX was not observed after the 5<sup>th</sup> cycle. Of the 99 cases of delayed excretion of MTX observed in the present study, 70 occurred during the first three cycles and 29 occurred after the 3<sup>rd</sup> cycle. Of the 11 cases of severe delayed excretion of MTX, 9 occurred during the first three cycles and 2 occurred after the 3<sup>rd</sup> cycle, but a significant difference was not observed. This finding may be related to the samples included in the studies: Of the 4291 cycles of chemotherapy in the present study, the ratio of 1–3 cycles and 4–11 cycles was 3020:1271, whereas in the study by Bacci *et al.*,<sup>[9]</sup> the ratio in the 4219 cycles they used was 2324:1895.

### Correlation between serum concentration of methotrexate and leucovorin rescue

Of the 11 patients with severe delayed excretion of MTX, 7 were found to have C<sub>24 h</sub> >50 μmol/L. The emergency

management plan was initiated immediately if  $C_{24\text{ h}} > 50$   $\mu\text{mol/L}$  was reported. The attending physician implemented treatment with close monitoring of the patient to prevent severe, irreversible damage. Simultaneously, the frequency and dose of leucovorin rescue were increased with continuous hydration and alkalization to maintain urine pH at 7–9 to eliminate MTX as quickly as possible. All 11 patients reached the standard of MTX excretion (serum concentration  $< 0.05$   $\mu\text{mol/L}$ ) within 7–28 days and proceeded to the next cycle of chemotherapy.

Treatments for delayed excretion of MTX include supportive care, appropriate dose of leucovorin, blood purification, and carboxypeptidase  $G_2$  (CPDG<sub>2</sub>) administration.<sup>[12,13]</sup> Hemodialysis, blood perfusion, and peritoneal dialysis can also be used for rescue of HD-MTX chemotherapy, but they can increase the risks of other complications (e.g., bleeding, infection) in cancer patients. Cavone *et al.*<sup>[14]</sup> reported on CPGD<sub>2</sub> use in the rescue from HD-MTX toxicity, but its safety has not been studied fully and, therefore, it is not used in China. The 11 patients all reached the standard for MTX excretion after sufficient leucovorin rescue without the need for invasive treatment, and potentially pathogenic complications were not observed.

Several studies have shown that the peak concentration in serum ( $C_{\text{max}}$ ) can be an indicator of the pharmacologic effect to predict the efficacy of HD-MTX treatment for osteosarcoma.<sup>[1,15,16]</sup> In general, a better prognosis can be obtained if the  $C_{\text{max}}$  4 h after administration can be controlled at 1000–1500  $\mu\text{mol/L}$ . In addition, given the incomplete understanding of the mechanism of the metabolism and clearance of MTX, the serum concentration of MTX can be an indicator for determination of delayed excretion and used to guide the dose and frequency of leucovorin administration. In the present study, patients with delayed excretion of MTX were found to have significantly higher values of  $C_{0\text{ h}}$  and  $C_{24\text{ h}}$  than patients with normal excretion. Furthermore, the  $C_{24\text{ h}}$  of patients with severe delayed excretion of MTX was significantly higher than that of patients with mild–moderate delayed excretion of MTX. Therefore, the  $C_{24\text{ h}}$  can be an indicator of the need to increase the frequency and dose of leucovorin.

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### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

1. Graf N, Winkler K, Betlemovic M, Fuchs N, Bode U. Methotrexate pharmacokinetics and prognosis in osteosarcoma. *J Clin Oncol* 1994;12:1443-51.
2. Mir O, Ropert S, Goldwasser F. Neoadjuvant chemotherapy with high-dose methotrexate in osteosarcoma. *Lancet Oncol* 2008;9:1198. doi: 10.1016/S1470-2045(08)70309-7.
3. Jaffe N. Recent advances in the chemotherapy of metastatic osteogenic sarcoma. *Cancer* 1972;30:1627-31.
4. Widemann BC, Balis FM, Kempf-Bielack B, Bielack S, Pratt CB, Ferrari S, *et al.* High-dose methotrexate-induced nephrotoxicity in patients with osteosarcoma. *Cancer* 2004;100:2222-32. doi: 10.1002/ncr.20255.
5. Lee KM, Lee HW, Kim SY, Lee HJ, Kim DH, Cho J, *et al.* Two pediatric osteosarcoma cases with delayed methotrexate excretion: Its clinical course and management. *Cancer Res Treat* 2011;43:67-70. doi: 10.4143/crt.2011.43.1.67.
6. Zhang W, Zhang Q, Zhao HT, Huang Z, Xu G, Zhu ZH, *et al.* Analysis of 2 cases of high-dose leucovorin rescue after methotrexate chemotherapy induced delayed excretion. *Chin Pharm* 2014;25:151-3. doi: 10.6039/j.issn.1001-0408.2014.02.19.
7. Bleyer WA. Methotrexate: Clinical pharmacology, current status and therapeutic guidelines. *Cancer Treat Rev* 1977;4:87-101.
8. Fleisher M, Schwartz MK. Measurement of anti-folate analogs. *Clin Chem* 1992;38:609-10.
9. Bacci G, Ferrari S, Longhi A, Forni C, Loro L, Beghelli C, *et al.* Delayed methotrexate clearance in osteosarcoma patients treated with multiagent regimens of neoadjuvant chemotherapy. *Oncol Rep* 2003;10:851-7. doi: 10.3892/or.10.4.851.
10. Holmboe L, Andersen AM, Mørkrid L, Slørdal L, Hall KS. High dose methotrexate chemotherapy: Pharmacokinetics, folate and toxicity in osteosarcoma patients. *Br J Clin Pharmacol* 2012;73:106-14. doi: 10.1111/j.1365-2125.2011.04054.x.
11. Wang YM, Sutow WW, Romsdahl MM, Perez C. Age-related pharmacokinetics of high-dose methotrexate in patients with osteosarcoma. *Cancer Treat Rep* 1979;63:405-10.
12. Cecyn KZ, Lee J, Oguro T, Petrilli AS, Bordin JO. Use of plasma exchange in methotrexate removal in a patient with osteosarcoma and acute renal insufficiency. *Am J Hematol* 2003;72:209-11. doi: 10.1002/ajh.10271.
13. Wang X, Zhang W. Treatment of delayed excretion of high-dose methotrexate. *Chin Hosp Pharm* 2012;32:1835-8. doi: 10.13286/j.cnki.chinhosp-pharmacy.2012.22.023.
14. Cavone JL, Yang D, Wang A. Glucarpidase intervention for delayed methotrexate clearance. *Ann Pharmacother* 2014;48:897-907. doi: 10.1177/1060028014526159.
15. Delepine N, Delepine G, Jasmin C, Desbois JC, Cornille H, Mathé G. Importance of age and methotrexate dosage: Prognosis in children and young adults with high-grade osteosarcomas. *Biomed Pharmacother* 1988;42:257-62.
16. Jaffe N, Gorlick R. High-dose methotrexate in osteosarcoma: Let the questions surcease – Time for final acceptance. *J Clin Oncol* 2008;26:4365-6. doi: 10.1200/JCO.2007.14.7793.