Korean Journal of Pediatrics Vol. 52, No. 5, 2009
DOI : 10.3345/kjp.2009.52.5.581
■ Original article : Clinical study ■

# Serum methotrexate level is inversely related to the outcome of osteosarcoma patients

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**= Abstract =**

**Purpose :** To evaluate the correlation between serum methotrexate (MTX) peak levels and clinical outcome of osteosarcoma, as well as to determine the correlation of these levels with the histologic response and event-free survival (EFS).

**Methods :** To maintain the homogeneity of the study population, we selected 52 patients with localized extremity osteosarcoma who had received two cycles of neoadjuvant chemotherapy consisting of high-dose (HD) MTX (12 g/m$^2$), cisplatin (100 mg/m$^2$), and doxorubicin (60 mg/m$^2$).

**Results :** Totally, 204 courses of HD MTX were administered. The serial MTX levels (mean±SE) at 4 h (peak), 24 h, 48 h, and 72 h were 1292.14±12.83 □M, 9.29±3.89 □M, 1.73±1.37 □M, and 0.58±0.44 □M, respectively. The peak MTX serum level was 1292.14±12.83 □M. Neither the continuous average MTX peak level nor the dichotomized MTX peak level was related to the histologic response. However, the patients with a high 24-h MTX level (3.4 □M) had a poor histologic response ($P$=0.044). An inverse relationship was observed between MTX levels and survival: the EFS was better in the patients with a mean MTX peak level of less than 1,400 □M ($P$=0.002) and mean 24-h MTX level of less than 3.4 □M ($P$=0.011).

**Conclusion :** The inverse correlation between the MTX level and the outcome is an unexpected finding. Further study on the pharmacokinetics of MTX is required to substantiate our findings and elucidate the mechanism involved. **(Korean J Pediatr 2009;52:581-587)**

**Key Words :** Osteosarcoma, Methotrexate (MTX), Serum level, Outcome

## Introduction

High dose methotrexate (HD MTX) is one of the major chemotherapeutic agents that are used for treating osteosarcoma[1]. The pharmacokinetics of HD MTX shows individual variability and this is influenced by many factors such as age, the hydration status and the concurrent use of nephrotoxic agents[2-4]. Individually monitoring the MTX serum levels enables appropriate alteration of the dose of leucovorin so as to avoid potential life threatening toxicities[5, 6].

Several studies have investigated the relationship between the MTX level and the histologic response or the patient outcome. The MTX serum level is supposed to have a linear relationship with tumor necrosis rate; however, inconsistent results have been reported. The earlier studies consistently advocated that a certain threshold MTX level (700 □M or 1,000 □M) must be achieved to attain a good histologic response[5, 7, 8]. However, the subsequent studies reported that the MTX level did not influence the histologic response [9-11]. Moreover, a recent study showed contradictory results that patients with very high MTX peak levels (above 1,500 □M) had poor outcome[12]. These results might partly be accounted for by the heterogeneity of the study populations and the chemotherapy protocols of the previous studies. Most of the previous studies have included both localized and metastatic disease. Moreover, the preoperative chemotherapy was not homogeneous with regard to the MTX dose and schedule.

In this retrospective study, we focused on maintaining the homogeneity of the study population; therefore, we selected

Received : 16 October 2008, Revised : 19 November 2008
Accepted : 5 December 2008
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those patients who met following criteria: 1) they had localized extremity osteosarcoma; 2) they received 2 cycles of neoadjuvant chemotherapy consisted of HD MTX, cisplatin and doxorubicin. We aimed to assess the correlation between the serum MTX levels and histologic response and outcome. We asked two questions: 1) Is the MTX peak level related to histologic response? If so, does a certain MTX level exist that must be achieved for obtaining a good histologic response? 2) In addition to the peak level, is the MTX excretion rate related to the outcome?

## Materials and Methods

### 1. Patient Selection

This was a retrospective study and patients who met the following criteria were eligible. They were: an age less than 40 years, AJCC stage II osteosarcoma[13], no history of treatment except for biopsy, primary tumor in an extremity, treated at our institute during 2003 to 2005, received 2 cycles of preoperative chemotherapy, including at least 3 HD MTX courses, and the event free patients having more than a 2-year follow-up period. During this period, a total of 109 patients were diagnosed as osteosarcoma and treated at our institute. Among them, 57 patients who failed to meet any of the abovementioned criteria were excluded. Reasons for exclusion are as follows: 12 cases were aged more than 40 years, 23 cases had lung metastasis at diagnosis, 1 was relapsed cases and 11 cases were in locations other than extremity. Eighteen cases were excluded because of chemotherapy. Three patients were treated only with HD MTX and 4 cases were treated with HD MTX, ifosfamide and bleomycin. And 5 patients were excluded because they had received 2 courses of HD MTX. Accordingly, the remaining 52 cases were analyzed.

Informed consent was required from all the patients or their legal guardians depending on the patient's age. The procedures for defining the extent of tumors included plain radiography and MRI of the primary tumor, a $^{99m}$Tc-methylene diphosphonate whole-body bone scan and a computed tomography scan of the chest. The AJCC stage of each patient was determined by measuring the absolute tumor length (ATL) on the MR images, and the absolute tumor volume was calculated according to the previous description by Bieling et al[14].

### 2. Chemotherapy Protocol

All the patients underwent two courses of preoperative chemotherapy followed by four courses of postoperative chemotherapy. A modified T10 chemotherapy protocol was used. Briefly, each course of chemotherapy consisted of HD MTX, doxorubicin, and cisplatin. HD MTX (12 g/m$^2$) was administered twice, on days 1 and 7. On day 14, 100 mg/m$^2$ cisplatin was given for 4 hours. Subsequently, 60 mg/m$^2$ doxorubicin was delivered for 48 hours. Definitive surgery was scheduled between weeks 10 and 12. The type of surgery (amputation or limb salvage) as well as the type of reconstruction after resection (prosthesis, resection arthrodesis, pasteurized autograft or temporary spacer) was chosen depending on the extent and location of the tumor, surgical margin, skeletal maturity and the presence of complicating factors such as pathological fracture or an adverse response to preoperative chemotherapy. The histologic response to preoperative chemotherapy was evaluated in all patients, following the criteria of Rosen et al. with less than 10% residual viable tumor indicating a good response, whereas more than 10% residual viable tumor indicated a poor response[15]. The postoperative chemotherapy was tailored according to the histologic response, i.e., good responders received the same therapy as the preoperative chemotherapy while the poor responders were switched to an ifosfamide-bleomycin-doxorubicin-cisplatin based protocol. The dosages of cisplatin and doxorubicin were the same as the preoperative doses, and ifosfamide (14 g/m$^2$) was infused continuously for 7 days along with the bleomycin (30 mg/m$^2$). The scheduled duration of chemotherapy was 29 weeks for the good responders and 33 weeks for the poor responders.

### 3. MTX Infusion and Analysis of the MTX Level

The patients were hydrated intravenously with 250 mL/m$^2$ of 5% glucose together with 100 mol/L $NaHCO_3$ and 20 mL KCl solution one hour before MTX infusion. The MTX was dissolved in 500 mL of $D_5W$ and this was infused over 4 hours. After MTX infusion, the patients were hydrated with the same fluid for a total of 1,500 mL/m$^2$ in the first 24 hours. The urine pH was monitored every 8 hours and the urine was alkalinized with $NaHCO_3$ to a pH$\geq$7. After 24 hours, the intravenous hydration was increased to a rate of 3,000 mL/m$^2$/day. Intravenous leucovorin rescue (15 mg/m2 every 6 hours) was begun 24 hours following the start of MTX infusion and the dose was adjusted according to the serum

MTX levels. Venous blood samples were taken at the end of the infusion (the peak value) and then every 24 hours, and the serum MTX levels were estimated by means of a FPIA (fluorescence polarization immunoassay) kit (Abbott Laboratories, Abbott Park, IL, USA). Leucovorin rescue, hydration and alkalinization were continued until a MTX level less than 0.15 ㎛M was achieved.

### 4. Statistical Analysis

We assessed the correlations between the MTX levels and the histologic response and event-free survival (EFS). The mean MTX levels according to the clinicopathologic variables were compared using independent Student's t-tests. The patients were divided into two groups using various cut-off values of peak and the 24-hr MTX levels. Chi-square tests were used to compare the histologic response and event in each group and to determine the significant univariate predictors. The event-free interval was defined as the date of diagnosis to the time of the last visit or the date when either metastasis or local recurrence was found. The survival analysis between the MTX levels and event was analyzed by the Kaplan-Meier method, and the difference was calculated with using the log-rank test. All calculations were performed with SPSS version 13.0 software (SPSS Inc, Chicago, IL), and $P$ values$<0.05$ was considered significant.

## Results

### 1. Patient Characteristics

The patient's characteristics are summarized in Table 1. The median age of the 52 patients was 16 years (range: 4-37 years). The location of tumor was at the distal femur in 22 cases (42.3%), the proximal tibia in 13 cases (25.0%), the proximal humerus in 10 cases (19.2%), the proximal fibula in 2 cases (3.8%) and elsewhere in 5 cases (9.6%). Fifty-one patients (98.1%) underwent limb salvage surgery, whereas 1 patient (1.9%) had amputation. The median follow-up duration of the 52 patients were 28 months (range: 13-53 months). There were no local recurrences and all the events were metastasis, with 9 cases in lung and 2 cases in bone. Mean event-free interval for the 43 patients who were without subsequent metastasis was 34.3±1.3 months and that for the entire cohort was 30.9±1.6 months. The 3-year EFS rate of the entire cohort was 84.5±5.1%.

### 2. The MTX Peak Level

While 48 patients completed 4 cycles of preoperative MTX, 4 patients received only 3 cycles of MTX because of delayed excretion and elevated liver enzymes. Thus, a total of 204 courses of high dose MTX were administered to 52 patients. Among the 204 courses of MTX, 168 courses (82.4%) achieved a peak level ≥1,000 ㎛M and the average value was 1292.14±12.83 ㎛M (mean±S.E). The MTX peak levels were not different according to the clinicopathologic variables. Those patients with a large tumor volume (≥150 mL) had a tendency to have a lower peak MTX level ($P$=0.074). There was no association between the continuous average MTX peak levels and the histologic response ($P$=0.895) and metastasis ($P$=0.196, Table 1). The serial MTX levels at 24-hr, 48-hr and 72-hr were (mean±S.E); 9.29±3.89 ㎛M, 1.73±1.37 ㎛M and 0.58±0.44 ㎛M, respectively.

### 3. Clinicopathologic Characteristics According to the MTX level

Using various cutoff values for MTX peak levels, we divided patients into two groups and compared with the histologic response and development of metastasis. None of

**Table 1.** Patient Characteristics and Methotrexate Peak Level

| Variables (n) | Peak MTX level (mean±S.E, ㎛M) | $P$-value |
|---|---|---|
| Age | | |
| ≤15 years (24) | 1301.05±59.82 | 0.829 |
| >15 years (28) | 1287.60±25.94 | |
| Gender | | |
| Male (31) | 1283.10±33.51 | 0.675 |
| Female (21) | 1309.63±58.42 | |
| Histologic subtype | | |
| Osteoblastic (37) | 1276.89±35.99 | 0.462 |
| Chondroblastic (4) | 1443.14±112.08 | |
| Fibroblastic (5) | 1240.49±70.49 | |
| Others (6) | 1343.05±112.86 | |
| AJCC stage | | |
| IIA (27) | 1310.89±43.31 | 0.567 |
| IIB (25) | 1275.36±43.84 | |
| ATV | | |
| ≤150 mL (38) | 1326.99±38.00 | 0.074 |
| >150 mL (14) | 1203.74±40.62 | |
| Histologic response | | |
| Good (19) | 1288.43±58.52 | 0.895 |
| Poor (33) | 1296.91±35.28 | |
| Metastasis | | |
| Yes (43) | 1380.99±79.60 | 0.196 |
| No (9) | 1275.56±32.84 | |

**Table 2.** Histologic Response and Development of Metastasis according to the Peak and 24-hr Methotrexate Level

| MTX level (□M) | | Number of patients | Good response (%) | *P*-value | Metastasis (%) | *P*-value |
|---|---|---|---|---|---|---|
| Peak | ≥1,100 | 42 | 35.7 vs 40.0 | 0.8 | 16.7 vs 20.0 | 0.80 |
| | ≥1,200 | 36 | 36.1 vs 37.5 | 0.92 | 19.4 vs 12.5 | 0.54 |
| | ≥1,274 (median) | 26 | 40.7 vs 32.0 | 0.51 | 25.9 vs 8.0 | 0.09 |
| | ≥1,300 | 22 | 36.4 vs 36.7 | 0.98 | 27.3 vs 10.0 | 0.10 |
| | ≥1,400 | 13 | 30.8 vs 38.5 | 0.62 | 46.2 vs 7.7 | 0.002 |
| | ≥1,500 | 10 | 30.0 vs 38.1 | 0.63 | 40.0 vs 11.9 | 0.04 |
| 24-hr | ≥2.10 (25 p) | 39 | 23.1 vs 76.9 | <0.0001 | 23.1 vs 0 | 0.06 |
| | ≥3.4 (median) | 26 | 23.1 vs 50.0 | 0.04 | 30.8 vs 3.8 | 0.01 |
| | ≥5.3 (75 p*) | 14 | 14.3 vs 44.7 | 0.04 | 42.9 vs 7.9 | 0.003 |
| | ≥12.3 (90 p) | 6 | 16.7 vs 39.1 | 0.28 | 33.3 vs 15.2 | 0.27 |

p, percentile

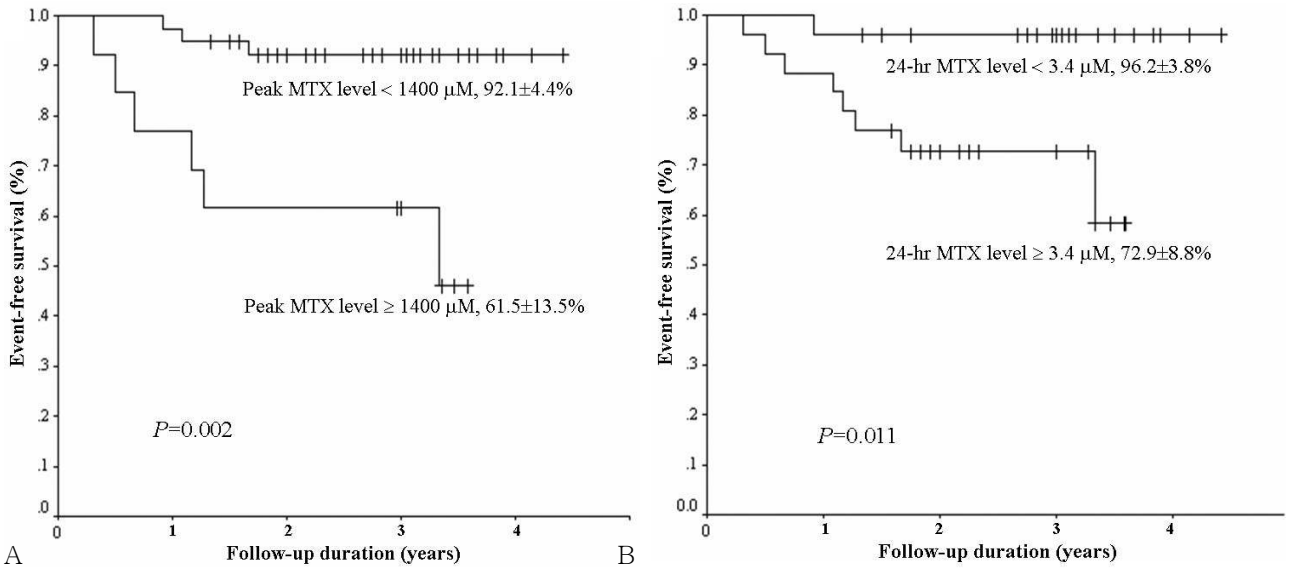


**Fig. 1.** Event-free survival (EFS) according to the peak and 24-h methotrexate (MTX) levels. (A) The three-year EFS is better in the patients with a mean MTX peak level of less than 1400 □M compared with the patients with a mean MTX peak level of greater than or equal to 1400 □M. (B) The patients with a mean 24-h MTX level of less than 3.4 □M fared better than the patients with a mean 24-h MTX level of greater than or equal to 3.4 □M.

the above-mentioned peak levels was related to good histologic response. On the contrary, too high peak level was related to poor outcome. Thirteen patients (25%) had achieved levels above 1,400 □M. Six of these patients (46.2%) developed metastasis, while 7.7% (3/39) of the patients with a MTX peak level <1,400 □M developed metastasis (*P*=0.002, Table 2).

Unexpectedly, 24-hr MTX level was related to the histologic response. Using various cutoff values for 24-hr MTX levels, we divided patients into two groups and compared its correlation with outcome. Patients with a 24-hr MTX level above the median value of 3.4 □M showed a poor histologic response (*P*=0.044) and were related to the development of metastasis (*P*=0.010, Table 2).

### 4. Survival Analysis According to the MTX level

The patients were divided into two groups using the cut-off values of the peak and 24-hr MTX levels. The cutoff value 1,400 □M and 3.4 □M were chosen based on the results on Table 2. An inverse relationship was observed between the MTX levels and survival: 1) The EFS of the patients whose mean MTX peak level <1,400 □M was better than the EFS of the patients with a mean MTX peak level 1,400 □M (92.1±4.4% vs. 61.5±13.5%, respectively, *P*=0.002), 2) the EFS of the patients whose mean 24-hr MTX level <3.4 □M was better than the EFS of the patients whose 24-hr level was ≥3.4 □M (96.2±3.8% vs. 72.9±8.8%, respectively, *P*=0.011, Fig. 1).

## Discussion

The association between the MTX peak levels and the outcome of osteosarcoma has been a matter of debate. Several studies have reported superior outcomes with using higher MTX doses rather than lower doses, and the MTX peak level was considered to be related with the histologic response[5, 7]. Because of the limited number of patients and the heterogeneity of the study population, the previous studies have produced conflicting results. To more precisely assess the relationship between the MTX level and the outcome, we selected patients with localized osteosarcoma who have received more than 3 cycles of HD MTX before definitive surgery. A MTX peak level above 1,000 □M was achieved in most of our patients (82.4%). It is intriguing that the patients with a high MTX level (both the peak and 24-hr levels) showed a shorter event-free survival. However, we could not find any positive association between the MTX peak level and the histologic response. In contrast, the 24-hr MTX level showed inverse correlation with the histologic response; that is, patients with a high 24-hr MTX level showed a poor histologic response.

Although this was a single-institution study, it has several limitations. First, this was a retrospective study and the patients were not randomized or controlled with respect to the treatment protocols, including the chemotherapeutic regimens and the type of operation. Second, the study population was small and the follow-up periods were not long enough. Last, we were not able to present detailed data about the pharmacokinetics of MTX, since the required software programs were not available in our institution.

The serum level of MTX is supposed to represent the drug concentration in tumor cells[5, 9]. Therefore, it is conceivable that a high serum MTX concentration might result in better tumor cell necrosis and possibly a good prognosis. However, this assumption might only be relevant when MTX plays a dominant role in killing tumor cells. In the era of combination chemotherapy, all the employed drugs play concerted roles in tumor cell necrosis[16]. Accordingly, the correlation between the MTX level and the histologic response is likely to be dependent on MTX's contribution to tumor cell necrosis. We reviewed previous studies, especially in the context of the proportion of MTX that was used in each protocol. We calculated the proportion of MTX using following simple formula: the number of MTX adminstrations/total number of administrations for all the drugs used in preoperative chemotherapy. For example, the proportion of MTX would be 100% in the MTX monodrug protocol. Delepine et al.[5] and Saeter et al.[7] who adopted the monodrug protocol, consistently found a close relationship between the MTX peak level and the histologic response[5, 7]. In the COSS-80 protocol, which was comprised of 4 courses of HD MTX and one course of both doxorubicin and cisplatin, the proportion of MTX was calculated as 67% (4/4+1+1)[9]. In this study, the MTX peak level ≥1,000 □M was related to a good histologic response. Contrastingly, in the successive COSS protocols and other trials, the proportion of MTX ranged from 28.6% to 50% and relationship between the MTX peak level and the histologic response was not found[9-11]. In the current study, the proportion of MTX was 50%. Our contradictory results (the inverse correlation between the MTX level and the outcome or the necrotic rate) were unexpected. Only one previous study supported our results[12]. These findings are counterintuitive and the causative mechanism for this association is unclear. One possibility is that an increased leucovorin dose in patients with very high peak and 24-hr MTX levels might have compromised the antitumor effect of MTX. Meyers et al. suggested that inferior survival of CCSG 782 to MSKCC T10 protocol might be attributable to the increased leucovorin dose[17]. Further studies are needed to clarify the optimal dose of leucovorin and its influence on tumor cells. Another possible explanation is that prolonged exposure to a high MTX concentration resulted in delayed delivery of the scheduled chemotherapy, thus reducing the intensity. In the present study, we evaluated the possible correlation between the MTX level and delay in the chemotherapy schedule. However, no correlation was found, since renal function was not impaired and delivery of next chemotherapy was not significantly delayed in patients with a high peak and 24-hr MTX levels (data not shown). Lastly, abnormal MTX pharmacokinetics may influence on the metabolism of other chemotherapeutic agents such as doxorubicin and cisplatin. CYP 3A4 isoenzyme family mediates the pharmacokinetic variability of doxorubicin [18-20]. A recent study reported that a high CYP3A4/5 expression in osteosarcoma cells could predict metastasis and poor prognosis[21]. At present, there is a limited evidence for drug interaction between MTX and doxorubicin; however, we presume that future studies concerned with the association between the pharmacokinetics and drug metabolism of MTX would give us some clues.

In conclusion, the patients with a high MTX level showed a shorter event-free interval and a poor histologic response. Further study about the pharmacokinetics of MTX is mandatory to substantiate our findings and elucidate the mechanism.

## 한 글 요 약

### 혈중 methotrexate 농도와 골육종 환자들의 치료결과의 연관성

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목 적 : 골육종의 항암치료 시 MTX의 혈중농도와 예후의 관련 여부에 대해 논란이 있어왔다. 저자들은 MTX의 혈중농도와 골육종의 치료결과간에 상관관계가 있는지 분석하였다.

방 법 : 원자력병원에서 2003년 1월부터 2005년 12월까지 골육종으로 치료를 받은 환자들 중 종양의 원발부위가 사지이고, 폐 전이가 없으며, 수술 전 고용량 MTX (12 g/m$^2$), cisplatin (100 mg/m$^2$), doxorubicin (60 mg/m$^2$)의 병합항암치료를 2회 받은 52명의 환자들을 선택하여 MTX 혈중농도와 항암치료에 대한 종양조직의 괴사율, 생존율의 상관관계를 분석하였다.

결 과 : 52명의 환자들에게 고용량 MTX 항암치료를 총 204회 시행하였다. MTX의 4시간, 24시간, 48시간, 72시간째 혈중농도는 각각 1292.14, 9.29, 1.73, 0.58 □M 이었다. 최고혈중농도(4시간째 혈중농도)는 종양조직의 괴사율과 관계가 없었지만, 24시간째 MTX 혈중농도가 3.4 □M 이상인 환자들이 그렇지 않은 환자들보다 종양조직의 괴사율이 불량하였다 (90%이상의 조직괴사율을 보이는 환자들의 비율, 23.1% 대 50%). MTX 혈중농도는 무사건 생존율과 역 상관관계가 있었다. 최고혈중농도 ≥1,400 □M (61.5% 대 92.1%), 24시간 혈중농도 ≥3.4 □M (72.9% 대 96.2%)인 환자들의 무사건 생존율이 낮았다.

결 론 : MTX의 혈중농도가 높은 환자들이 그렇지 않은 환자들보다 치료결과가 불량하였다. 향후 골육종 환자들에서 MTX 약물역동학에 대한 연구를 시행하여 본 연구결과의 기전을 밝혀야 할 것이다.

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