Original Article

A survival analysis and treatment outcomes of 131 cases of osteosarcoma treated with high-dose methotrexate multidrug chemotherapy

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Received November 19, 2015; Accepted March 29, 2016; Epub May 15, 2016; Published May 30, 2016

Abstract: Objective: To evaluate the effect and security of high-dose methotrexate (HD-MTX) multidrug first-line chemotherapy on primary osteosarcoma in regions near the knee joint, as well as its effect on the patients survival and the prognosis-related clinical features. Methods: A retrospective analysis was performed on the data collected from the Hangzhou Third Hospital during June 2007 to June 2012. These patients were treated with HD-MTX/HD-MTX/DDP (cisplatin)/ADM (doxorubicin) and/or HD-MTX/IFO (Ifosfamide)/DDP/ADM, and surgery respectively. The treatment effects were followed up. Kaplan-Meier method was adopted for survival analysis and the survival curve was graphed. Log-Rank test was applied for single factor analysis while COX’s Proportional Hazard Model was used for multi-variate analysis. Result: The average follow-up duration was 51.14 months. The follow-up rate was 91.6%. 117 cases received neoadjuvant chemotherapy. No case had complete response (CR). Partial response (PR), stable disease (SD) and disease progression (PD) were seen in 82, 20 and 15 patients respectively. The objective response rate (RR) was 70.08% (82/117). The disease control rate (DCR) was 87.18% (102/117). The 3-year event-free survival, overall survival, local recurrence and distant metastasis were 67.94%, 83.21%, 6.11% and 19.08%, respectively. Improvements has been seen in 103 patients who completed 4-cycle chemotherapy, whose 3-year event-free survival, overall survival, local recurrence and distant metastasis were 72.82%, 84.47%, 2.91% and 17.48%. Standard chemotherapy can significantly (P<0.05) improve patients prognosis including 3-year survival, event-free survival, 3-year local recurrence and distant metastasis. Univariate analysis showed that survival is significantly related to age, pathologic fracture, standard chemotherapy, histological response, orthopedic surgical options, and distant metastasis (P<0.05), among which age, pathologic fracture and histological response are independent factors that related to prognosis. Conclusion: HD-MTX multidrug therapy has both satisfying short-term and long-term effects. The side effect was controllable. Patients following this regimen showed a good level of compliance. Therefore, this regimen is highly recommended. Having an optimized and new regiment through multi-discipline collaborations is the key to a better osteosarcoma treatment.

Keywords: Methotrexate, combination chemotherpy regimen, osteosarcoma, knee joint adjacent region, survival analysis

Introduction

Osteosarcoma is the most common malignant primary bone tumor. It accounts for 35% of all malignant bone tumors. The estimated population with osteosarcoma is 2-3 in every million. The median age of osteosarcoma population is 16 to 20 years [1]. In China, 6000-7000 cases were added to this population each year [2]. Osteosarcoma has imposed a great threat to children and young adult. Osteosarcoma is highly malignant and easy to metastasize, with lung being the most common metastasized site. Lung metastasis was found in approximately 85% to 90% patients at the time when the osteosarcoma was affirmatively diagnosed [3]. The majority will die within 2 years due to lung metastasis. Even for the patients who undertook comprehensive treatments such as surgery and chemotherapy, 32% to 46% of them still experienced lung metastasis. The 5-year survival of the patients in stage IV is below 20% [4]. Thus lung metastasis is the root-ed cause leading to failing treatment and low survival rate.

Our department has adopted the HD-MTX multidrug regimen in conjunction with surgical
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treatment and achieved favorable outcomes. In considering that osteosarcoma frequently occurs in distal Femur and proximal Tibia around the knee joint, we retrospectively studied 131 cases of osteosarcoma, who received surgical treatment and follow-up for over 3 years. The report is as follows:

Patients and methods

Patient inclusion criteria

No former osteosarcoma history; Surgical resection evidently showing primary osteosarcoma in distal Femur and proximal Tibia; Treated for the first time; Absence of metastasis in primary diagnostic report; In stage I and II by Enneking Staging System; No other disease that may affect the chemotherapy; Having a complete set of raw data and follow-up data.

General information

192 patients with primary osteosarcoma were admitted into our department during June 2007-June 2012. 131 cases were included in this analysis, which comprises 81 males and 50 females. The age range was 7 to 62 years with the mean was 19.97 years and the median was 16 years. 94 cases had osteosarcoma in distal Femur (Left 48 cases, Right 46 cases). 37 cases had in proximal Tibia (Left 17 cases, Right 20 cases). 22 cases had pathologic fracture at the onset and during the treatment process. 78 cases had normal serum alkaline phosphatase (AKP) level, 53 cases were found elevated. Neuron-specific enolase (NSE) was found normal in 64 patients and increased in 67 patients. Pathological typing: 59 osteoblastic cases, 37 chondroblastic cases, 17 fibroblastic cases and 18 other cases. All the patients were followed up for more than 3 years, with the longest duration being 96 months and the shortest being 51.14 months. The lost-to-follow-up rate was 8.4%.

Chemotherapy regimen [5]

A regimen: MTX-MTX-DDP-ADM: MTX: 12 g/m² (children), 8 g/m² (adult), on day 1 and day 8; CDDP: 90-120 mg/m², on day 15; ADM: 60 mg/m², on day 17, repeat the administration in every 31 days.

B regimen: MTX-IFO-DDP-ADM: MTX: 12 g/m² (children), 8 g/m² (adult), on day 1; IFO: 1.8-2 g/m², from day 8 to 12; Mesna: 360 mg/m², injected with IFO, followed by injections in 5, 8, 11, 14, 17 hours; CDDP: 90-120 mg/m², on day 19; ADM: 60 mg/m², on day 21 repeat the administration in every 35 days.

Treatment plan

Neoadjuvant chemotherapy: 2 cycles of regimen A would be prescribed after biopsy confirmation. Post-surgical chemotherapy: 2 more cycles of regimen A would be prescribed given the tumor necrosis rate was larger than 90%. Otherwise, 2 cycles of regimen B would be prescribed. Postoperative adjuvant chemotherapy: 4 cycles of regimen A following the operation.

Treatment outcome and toxic effect evaluation

The treatment effect was evaluated based on RECIST guideline 1.1, which classifies patients into four levels including complete response (CR), partial response (PR), stable disease (SD) and progression disease (PD). The response rate (RR) was CR+PR. Disease control rate (DCR) was RR+SD. Treatment outcome was assessed every 8 weeks or every 2 cycles. Toxic side effect was graded by following guideline NCI-CTC 4.0. Grading ranges from grade I-IV. The evaluated toxic side effects included myelosuppression, mucositis, liver damage, GI tract reaction, cardiotoxicity, neurotoxicity, and skin allergies.

Key indice

Major indice: Event-free survival (EFS): The survival rate quantifying those who lived without recurrence and metastasis, from the time when they received operative treatment to the point they were followed up at year 1, 2 and 3. Overall survival (OS): The survival rate quantifying those who lived from the time when they received operative treatment to the point they were followed up at year 1, 2 and 3, including all tumor carriers.

Minor indice: Histological response rate: the most objective evaluating standard for neoadjuvant treatments is the histological responses of osteosarcoma towards chemotherapeutic drugs. In details, histological responses include clinical symptom assessment, radiology images, laboratory tests and tumor necrosis rate. A tumor necrosis rate larger than 90% is regarded as a good response. A bad response,
Table 1. Toxic side effect of HD-MTX multidrug chemotherapy (n=131)

<table>
<thead>
<tr>
<th>Toxic side effect</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelosuppression</td>
<td>17 (13.0)</td>
<td>45 (34.4)</td>
<td>42 (32.1)</td>
<td>27 (20.5)</td>
<td>131 (100.0)</td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>40 (30.5)</td>
<td>20 (15.3)</td>
<td>9 (6.9)</td>
<td>7 (5.3)</td>
<td>76 (58.0)</td>
</tr>
<tr>
<td>Nauseas, Vomiting</td>
<td>41 (31.3)</td>
<td>57 (43.5)</td>
<td>21 (16.0)</td>
<td>12 (9.2)</td>
<td>131 (100.0)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>13 (9.9)</td>
<td>6 (4.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>19 (14.5)</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>37 (28.2)</td>
<td>42 (32.1)</td>
<td>3 (2.3)</td>
<td>0 (0.0)</td>
<td>82 (62.6)</td>
</tr>
<tr>
<td>Hematuria, Proteinuria</td>
<td>10 (7.6)</td>
<td>3 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>13 (9.9)</td>
</tr>
<tr>
<td>Skin allergies</td>
<td>16 (12.2)</td>
<td>4 (3.1)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>21 (16.0)</td>
</tr>
<tr>
<td>Cardiotoxicity</td>
<td>5 (3.8)</td>
<td>3 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>8 (6.1)</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>6 (4.6)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>7 (5.3)</td>
</tr>
</tbody>
</table>

Statistical analysis

All data were processed with SPSS 20.0. A p value less than 0.05 was considered significant. Univariate survival analysis was conducted with Kaplan-Meier method and Log-Rank test. Multivariate analysis was carried out on factors found significant in univariate analysis by using COX’s Proportional Hazard Model, for the purpose of screening independent factors that affect prognosis.

Result

Treatment outcome

14 cases were not evaluated for short-term treatment outcome because these 14 cases were treated surgically and did not participate in neoadjuvant chemotherapy. The rest 117 cases all participated in neoadjuvant chemotherapy. We had 0 case CR, 82 PRs, 20 SDs and 15 PDs. The objective response rate and disease control rate were 70.08% (82/117) and 87.18% (102/117), respectively. The 3-year overall survival rate, event-free survival rate, local recurrence and distant metastasis were 83.21%, 67.94%, 6.11% and 19.08%.

Follow-up result

Event-free survival rate: In 1-year follow-up, 118 out of 131 cases survived. The rate was 90.08%. In 2-year follow up, 97 survived. The rate was 74.05%. 3-year survival rate was 67.94%, meaning 89 cases survived. 103 completed at least 4 cycles. Among those who completed 4+ cycles, 96 survived the 1st year tumor-free (93.20%), 84 lived longer than 2 years tumor-free (81.55%), and 75 lived through year 3 tumor-free (72.82%) (P<0.05).

Overall survival rate: Among 131 cases, 124 cases (94.66%) survived the 1st year; 116 cases (88.55%) survived 2 years; 109 cases (83.21%) survived 3 years. 103 cases completed 4+ cycles. 1, 2 and 3 years survival rate for those who completed 4+ cycles were 98.06% (101/103), 91.26% (94/103) and 84.47% (87/103) (P<0.05), respectively. 83 cases lived 4+ years, of whom 83.13% completed 4+ cycles.

Histological responses: 102 cases showed alleviated local pain, reduced or vanished lump and increased joint activity. Plain X-ray showed increase in tumor ossification and calcification. MR showed a significant reduced soft tissue lump size around the tumor. The swelling disappeared and the edge between the tumor and
Figure 1. Kaplan-Meier analysis for prognosis related factors. A. Age; B. Pathologic fracture; C. Standard chemotherapy; D. Histological response; E. Orthopedic procedures; F. Distant metastasis.
adjacent tissue became distinguishable. Physical measure such as circumferences of osteosarcoma lump apparently decreased. AKP and LDH level dropped or return to normal level. The effective rate of neoadjuvant treatments was 87.18%.

Local recurrence: 3 cases (2.29%) among 131 reported local recurrence in 1-year follow-up; 7 local recurrence cases (5.34%) in 2-year follow-up; 8 local recurrence cases (6.11%) in 3-year follow-up. 103 cases completed 4+ cycles. Among these 103 cases, 3 cases (2.91%, P<0.05) had local recurrence in 3-year follow-up.

Distant metastasis: 10 cases (7.63%, 12 lung metastasis) among 131 reported distant metastasis in 1-year follow-up; 22 distant metastasis cases (16.79%, 22 lung metastasis) in 2-year follow-up; 25 distant metastasis (19.08%, 25 cases lung metastasis, 1 case complicated with liver, bone and lymph node metastasis) in 3-year follow-up. 103 cases completed 4+ cycles. Among these 103 cases,
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18 cases (17.48%, \(P<0.05\)) had lung metastasis in 3-year follow-up.

Limp-salvage rate: 29 cases among 131 received amputation (including disarticulation), among which 7 cases were post-limp-salvage secondary amputation. Primary limp-salvage rate was 83.21% and the overall salvage rate was 77.86%.

Serology: 53 showed elevated AKP level before chemotherapy. 47 recovered to normal level after neoadjuvant chemotherapy. The rest 6 cases also recovered to normal level after operations. Among 67 cases had increased NSE, 58 cases returned to normal.

Analysis of prognosis

Univariate analysis: Kaplan-Meier method and Log-rank test showed that the survival is significantly related to age, pathologic fracture, standardized chemotherapy, operational options and distant metastasis (\(P<0.05\) for each factor, survival curve shown in Figure 1A-F). Gender, primary site, early diagnosed AKP level and local recurrence were not significant to prognosis \(P>0.05\) (Please see Table 2).

Multivariate analysis: 6 factors including age, pathologic fracture, standardized chemotherapy, histological response and surgical procedure options and metastasis were fed into Cox proportional hazard model. The result showed that age, pathologic fracture, histological response and distant metastasis were significantly associated with prognosis (see Table 3).

Discussion

The region adjacent to the knee joint is the most common site that develops osteosarcoma. 60-70% osteosarcoma occurs in that region [6, 7]. This condition can also develop in children and young adults. The current clinical solution focuses on the life prolongation, limb preservation and reducing negative mental impact. Neoadjuvant and postoperative adjuvant chemotherapy, which give coverage throughout the process of osteosarcoma treatment, have provided a solid guarantee for positive long-term result of limp-salvage operations. Currently the most effective first-line chemotherapy drugs [8] are HD-MTX, IFO, ADM and DDP. Osteosarcoma Research Center worldwide has proposed some famous protocol [9-11], such as Rosen’s T protocol and COSS protocol. However, the best combination of chemotherapy drugs and the best timing for treatment is still subject to debate.

In this study, 131 patients were treated with HD-MTX multidrug regimen. We had 70.08% response rate, 87.18% DCR and 83.21% limb-salvage rate. 3-year event-free survival, overall survival, local recurrence and distant metastasis were 67.94%, 83.21%, 6.11% and 19.08%, respectively. 103 patients undertook standard chemotherapy, whose 3-year event-free survival, overall survival and local recurrence and distant metastasis were 72.82%, 84.47%, 2.91% and 17.48%. Standard chemotherapy can significantly improve patients prognosis including overall survival, event-free survival, local recurrence and distant metastasis (\(P<0.05\)). These 3-year survival rate and event-free survival rate were considered leading rates on the national level [12]. This has proven that it is safe to use HD-MTX multidrug chemotherapy. A complete 4-cycle chemotherapy can effectively enhance the overall chance to survive and to survive in a tumor free state, reduce the likelihood of reoccurrence and the undesirable metastasis to distant sites. This study will provide great ref-

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Table 3. COX proportional hazard regression

<table>
<thead>
<tr>
<th></th>
<th>Regression Coefficient</th>
<th>Standard error</th>
<th>Wald (X^2) value</th>
<th>Degree of freedom</th>
<th>(P) value</th>
<th>OR value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-1.136</td>
<td>0.419</td>
<td>7.347</td>
<td>1</td>
<td>0.007*</td>
<td>0.321</td>
<td>0.141 - 0.730</td>
</tr>
<tr>
<td>Pathologic fracture</td>
<td>-1.686</td>
<td>0.453</td>
<td>13.862</td>
<td>1</td>
<td>0.000*</td>
<td>0.185</td>
<td>0.076 - 0.450</td>
</tr>
<tr>
<td>Orthopedic procedure</td>
<td>0.302</td>
<td>0.442</td>
<td>0.466</td>
<td>1</td>
<td>0.495</td>
<td>1.352</td>
<td>0.569 - 3.215</td>
</tr>
<tr>
<td>Standard chemotherapy</td>
<td>0.276</td>
<td>0.478</td>
<td>0.333</td>
<td>1</td>
<td>0.564</td>
<td>1.318</td>
<td>0.516 - 3.364</td>
</tr>
<tr>
<td>Histological response</td>
<td>0.987</td>
<td>0.419</td>
<td>5.544</td>
<td>1</td>
<td>0.019*</td>
<td>2.683</td>
<td>1.180 - 6.102</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>1.212</td>
<td>0.434</td>
<td>7.819</td>
<td>1</td>
<td>0.005*</td>
<td>3.361</td>
<td>1.437 - 7.861</td>
</tr>
</tbody>
</table>

Note: *\(P<0.05\) was considered statistically significant.
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erencing value in improving the overall osteosarcoma treatment.

Univariate analysis has shown that gender, primary site, serum AKP level and local recurrence provide no significant information to prognosis. This condition is more likely to develop in men, with a likelihood ratio 1.5-2. But in consistent with most literatures [13, 14], no significant gender difference in prognosis was found. Due to the unique anatomical structure of Tibia, bone tumors on Tibia are more likely to be identified at an early stage. As a result, the median survival for patients with femur osteosarcoma is longer than those with tibia tumors, yet such a result is not significant (P=0.128). Patients with tibia tumor included in this study only accounts for 28.2%. Significant findings might be achieved by increasing sample sizes and increasing the follow-up duration. Serum AKP level is sensitive in reflecting osteogenesis. Chemotherapy prevents the cancerous cell from doing damages to normal bones, brings pathological osteogenesis to a halt and eventually leads to a reduced level of serum AKP. Bacci et al.'s investigation showed that preoperative AKP level augmentation is significantly negatively correlated to survival. In our study, among 53 cases with augmented AKP level, 47 cases' level were restored to normal after neoadjuvant chemotherapy, implying good chemotherapy responses and relatively good prognosis. However, speaking from the overall data, AKP level at early diagnosis is not significantly associated with survival. This might be attributed to high physiological AKP level in children patients, whose bone development is active. These children constitute a significant portion of our data sample and this might be the reason of our insignificant result. Other studies [16] suggested that an AKP isozymes bone alkaline phosphatase (BALP) can serve as a more accurate predictor for osteosarcoma development and recurrence. Unfortunately, due to the complicated detection procedure and high costs, BALP as an osteosarcoma predictor is not widely adopted. Limb-salvage is the primary option for children and young adults with early stage osteosarcoma. But the chance of recurrence is unavoidable due to the existing tumor stem cells and drug resistance. Out of 8 recurrence cases in our study, 7 had timely secondary amputation and resulted in acceptable prognosis. Their survival is not significantly different from those without recurrence.

Our study found that survival rate was significantly related to age, pathologic fracture, standardized chemotherapy, histological response, surgical options and distant metastasis (P<0.05). Patients younger than 14 years had a significantly higher mortality rate compared to who older than 14 years (P=0.009) and this factor significantly affect patients' prognosis (P=0.007). This finding may contradict with the finding in other reports [18, 19], but it bears significant clinical meaning to patients with osteosarcoma. That is for patients at a relatively younger age and having a poor chemotherapy response, a timely and thorough surgical procedure or an alternative chemo regimen is needed to capture the best treatment time frame. In consistent with Xiaohui Nu's report [20], pathologic fracture is a critical factor affecting prognosis. Bone fracture could cause tumor cells to spread and infiltrate to the tissue in the vicinity and further result in local colonization or metastasis through the circulating system. Through this mechanism, bone fracture leads to a poorer prognosis. 7 cases in the study had local recurrence after limb-salvage and chemotherapy. An acceptable prognosis was achieved after secondary amputation. Additionally, 22 cases had poor prognosis after primary chemotherapy and a failed limb-salvage procedure (P=0.032). This confirmed that a good histological response is one of the markers for good prognosis. This also implied that effective neoadjuvant treatment is as good as amputation procedures in determining long term outcomes. The meta-analysis conducted by Bramer et al. [21] showed that histological response to pre-operative chemotherapy is a reliable independent risk factor in prognosis prediction. Chemotherapy evaluation standards include the treatment completeness, drug dose and efficacy. All these play important roles in improving prognosis of osteosarcoma patients. HD-MTX/HD-MTX/DDP/ADM and/or HD-MTX/IFO/DDP/ADM regimens were adopted in the treatment. Such a treatment plan has achieved satisfying short-term and long-term outcomes, manageable side effects and good patient compliance. Thus it is worthy to recommend. Distant metastasis is also an important prognostic index. It was reported [22] that patients in stage III with good histological responses had a better prognostic 3-year survival compared to patients in stage II with poor histological responses. However, our study still sh-
owed that distant metastasis is related to 3-year survival. If lesions cannot be completely removed, multi-cyclic chemotherapy treatments can only serve to extend the survival and the patients will ultimately die from either recurrence or toxic side effects. Thus it is predictable that distant metastasis becomes an increasingly significant matter to survival as the treatment goes by.

To summarize, osteosarcoma treatment should be based on a complete surgery and standardized chemotherapy. The key to osteosarcoma treatment improvement is multi-disciplinary collaboration, in keeping in mind that the ultimate goal is better prognosis and life quality.

Acknowledgements


Disclosure of conflict of interest

None.

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