Tissue necrosis after chemotherapy in osteosarcoma as the important prognostic factor

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ABSTRACT

الأهداف: من أجل تحديد الاستجابة النسيجية للعلاج الكيميائي قبل العملية الجراحية لنسبة من نخر الورم وتقييم الصلة بين الاستجابة النسيجية والنتائج الورمية.

الطريقة: تمت معالجة، وإجراء عملية الاستئصال، وتلقي العلاج الكيميائي قبل وبعد العملية لثمانين مريضاً مصاباً بالورم العظمي اللحمي، بمستشفى شافا يحيان – إيران، خلال الفترة ما بين عام 2003م وحتى عام 2005م. تم فحص كل العينات المستأصلة التي أجريت لها العملية الجراحية، وتحديد درجة الاستجابة النسيجية للعلاج الكيميائي قبل العملية. نخر من الدرجة الأولى لنسبة 50% من الورم أو أقل، نخر من الدرجة الثانية لأكثر من 50%، ولكن اقل من نسبة 90%، الدرجة الثالثة من النخر لأكثر من 90%.

النتائج: كانت الفترة الفعلية للمتابعة للمرضى الناجيين الذين استمرت حالتهم خالية من المرض 1044 يوماً. الاستجابة النسيجية للعلاج الكيميائي قبل العملية (p=0.016)، وكانت أكثر التنبؤات أهمية للنجاة الخالية من المرض. بلغ معدل حدوث النجاة الخالية من المرض لفترة قصيرة لثمانين مريضاً 86% (69 مريض) عند الشهر الثاني عشر، و50% عند الشهر الرابع والعشرون (24 مريض)، و21% (5 مرضى) عند الشهر الأربعون، مع نجاة خمسة مرضى لمتوسط 1096 يوماً.

خاتمة: تعتبر الاستجابة النسيجية للعلاج الكيميائي قبل العملية عامل تنبؤ سريري مهم لنتيجة علاج العملية للورم العظمي اللحمي. يجب استعمال هذا العامل من أجل تحديد المرضى الذين لديهم خطورة عالية من انتشار المرض، حيث أن مثل هؤلاء المرضى قد يكونوا مرشحين للمزيد من للعلاج المكثف.

Objective: To determine the histological response to preoperative chemotherapy of the percentage of tumor necrosis, and to assess the relationship between the histological response and the oncological result.

Methods: Eighty patients with osteosarcoma were managed with preoperative and postoperative chemotherapy and operative resection at Shafa Yahyaeeyan Hospital, Tehran, Iran between 2003-2005. Sections of each operative specimen were examined, and the histological response to chemotherapy was graded. Grade 1 indicated necrosis of 50% of the tumor or less; grade 2, necrosis of more than 50% yet less than 90%; grade 3, necrosis of more than 90%.

Results: The mean duration of the follow-up of the surviving patients, who were continuously free from disease was 1044 days. The histological response to preoperative chemotherapy (p=0.016) was the most important predictor of event-free survival. The rate of event-free short-term survival for the 80 patients entering this study was 86% (69 patients) at 12 months, 50% (24 patients) at 24 months, and 21% (5 patients) at 40 months, with 5 patients surviving for a median of 1096 days.

Conclusion: The histological response to preoperative chemotherapy is an important clinical predictor of the result of operative treatment of osteosarcoma. This indicator should be used to identify patients who are at high risk for metastasis, as such patients may be candidates for more intensive or novel therapy.

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Osteosarcoma is the most frequent primary malignant bone tumor, originating often in the metaphyses of long bones of adolescents.[1] The interdisciplinary treatment concept including the aggressive polychemotherapy, has lead to dramatic prognostic improvement in young patients with localized extremity disease, with relapse free survival rates of approximately 50-80% as reported by specialized centers or multicentric groups.[1-16] The uniform treatment concept of pre- and postoperative chemotherapy in combination with aggressive surgery has formed the basis of all consecutive neoadjuvant study protocols since 1980.[16]

Methods. Eighty patients who met the inclusion criteria were enrolled in this study between 2003-2005. They were managed for osteosarcoma at Shafa Yahyaeeyan Hospital, Tehran, Iran received similar chemotherapy protocol. Patients with osteosarcoma as a secondary malignancy or recurrent disease, and patients who had both irradiation and chemotherapy, were all excluded from the study. As radiation therapy plus chemotherapy before operation increases the extent of tissue necrosis, and it biases the comparisons of patients getting chemotherapy, plus radiation therapy with chemotherapy alone, so all patients who received radiation therapy plus chemotherapy, were all excluded from the study. The follow up of the patients who were alive and free from disease was between 200-1200 days with mean follow up of 1044 days. The mean age of diagnosis of the disease was 15.6 years (standard deviation 3.7), range from 8-24 years. There were 6 upper limb and 74 lower limb involvements of tumor, and 41 cases had proximal limb tumor.

The initial evaluation of all patients included the recording of the medical history, physical examination, and hematological studies, procedures used to define the extension of the primary tumor including conventional radiography in all cases. A negative chest x-ray and negative TC-99 methylene diphosphonate bone scan are required for exclusion of primary metastasis. During the follow up, radiogram of the chest, and the primary tumors were to be repeated at regular intervals specified in the respective treatment protocol. The size of the tumor was estimated by measuring its largest available dimension on radiographic image that had been made before treatment, or by determining the maximum size of the restricted tumor with pathological examination. Pre- and postoperative chemotherapy were to be given to all patients. All protocols included high doses of methotrexate with leucovarin rescue, in addition, doxorubicine, cisplatin isophosphamide, and bleomycin, cyclophosphamide, and dactinomycin were used in varying combinations. The scheduled duration of chemotherapy ranged from 24-38 weeks. Definitive surgery was scheduled to take place between ninth and seventeenth weeks.[16] The type of surgery was specified as amputation, wide resection, and marginal resection. This study was approved by the Ethical Committee of Iran University of Medical Science, Iran.

Histological studies. The histological diagnosis of osteosarcoma was based on the results of open biopsy or needle biopsy. Hematoxylin and eosin as well as periodic acid Schiff were used to detect the tissue sections.

Determination of the percentage of tumor necrosis. Each resected specimen was bisected, and the perimeter of the tumor was defined grossly. The entire area of the bisected tumor was partitioned into blocks of tissue averaging 2 cm^2 en face. All areas of the specimen, including the medullary canal, residual cortical bone, and surrounding soft tissue were included, and histological slides were prepared from these blocks. The extent of the necrosis was graded relative to the percentage of residual viable tumor in a semiquantitative manner, described by Huvos[17] and Rosen et al.[18] A grade one histological response was characterized by little or no necrosis (involving 50% of the tumor or less), a grade 2 response by necrosis more than 50% yet less than 90% of the tumor, and a grade 3 response by only scattered foci of viable tumor cells (necrosis more than 90%). The histological response to preoperative chemotherapy was determined retrospectively by the same pathologist in a blind fashion. Statistical analysis was performed using SPSS Version 15 for windows.

Results. The rate of event free short-term survival for 80 patients entering study was 86% (69 patients) at 12 months, 50 % (24 patients) at 24 months, and 21% (5 patients) at 40 months ,whereas 5 surviving patients were followed for median of 1096 days (**Figure 1**). In our study, we had 50 males, and 30 females patients, the median age of admission was 15 years (7-47 years). Femur was the most frequent location of tumor with 42 cases (53%), and tumors of tibia was 32 cases (40%), and humerus was 6 cases (7%). The mean was 12.7 cm and median was 14 cm diameters of tumors (8-25 cm). We had 11 amputations, 65 wide resections, and

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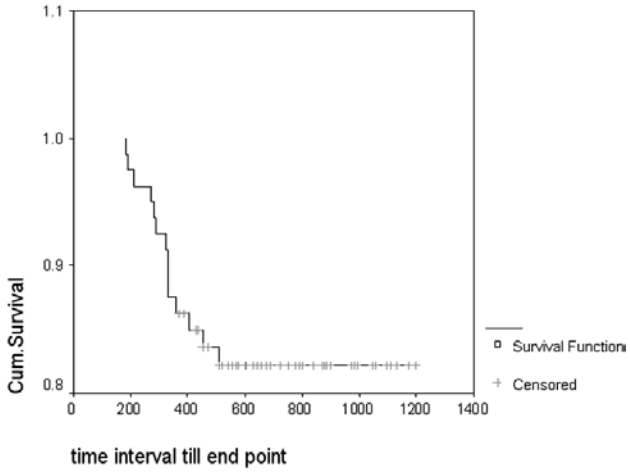

Figure 1 - The survival of all patients (the end point is death or recurrence of disease).

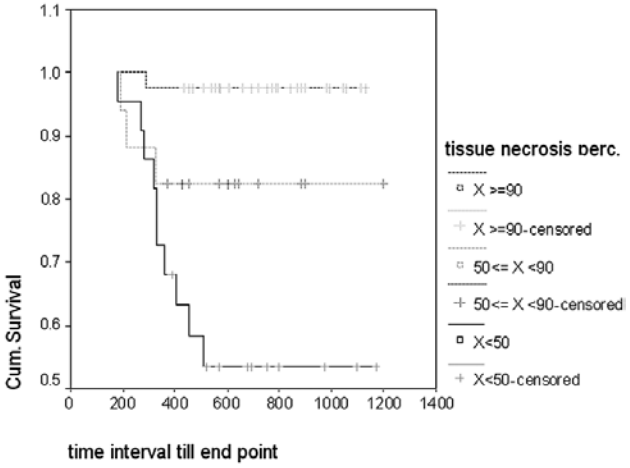

Figure 2 - The survival of patients according to the percent of tissue necrosis.

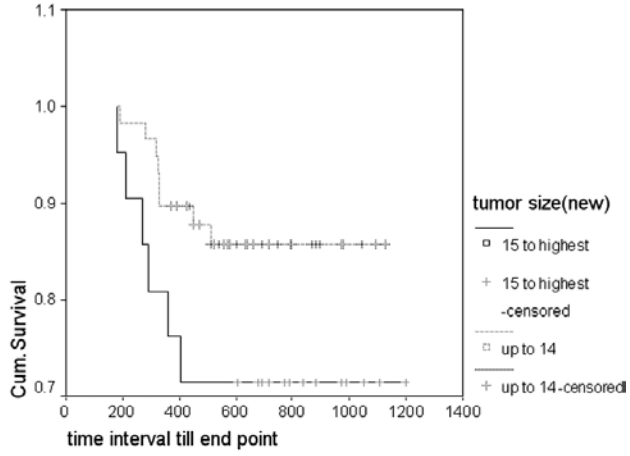

Figure 3 - The survival of patients according to the categorized size of tumor.

Table 1 - Logistic regression model showing the effect of tissue necrosis on survival.

Variables	*P*-value	Odds ration
Tissue necrosis	0.016*	16.67
Operation kind	NS	3.0
Metastasis	NS	2.2
Constant	NS	1.2

*Significant <0.05, NS - non significant

4 marginal resections. Most of the local recurrences (72%, 18 out of 25 patients) manifested within 2 years. Only 4 patients were followed more than 3 years in our short-term study.

Histological response to preoperative chemotherapy. Histological analysis of the 80 primary tumors after preoperative chemotherapy showed that 41 (51.3%) had nearly completed response (grade 3 necrosis of 90-99% of the tumor). In contrast, 22 tumors (27.5%) demonstrated little or no response (grade one necrosis of 50% or less), and 17 tumors (21.3%) exhibited a moderate response (grade 2 necrosis of more than 50% yet less than 90%). We did not find any significant association between a good response (less than 10% possible tumor) to chemotherapy, and probable factors affecting tissue necrosis such as age, gender, and tumor size. (p>0.05, chi squared test).

Factors affecting event-free survival. The grade of histological response was strongly associated with event-free survival (p=0.00001, **Figure 1**). The difference in event-free survival of the patients who had good (grade 3) histological response, and those who had grade one p=0.00001, or grade 2 p=0.0004 response was significant, however, difference in event-free survival of grade one and 2 was not significant (p=0.07). The rate of event-free survival at 3 years was 3-22 who had grade one necrosis, 10 of 17 who had grade 2 necrosis, and 40 of 41 who had grade 3 necrosis (**Figure 2**).

Eighty tumors were classified into 2 groups according to whether one dimensional size of the tumor (the median measurement) was 14 cm or less, or greater than 14 cm (range of size, 8-25 cm). At 3 years, the rate of short term event-free survival was 69% (40 out of 58 patients) for patients who had a small tumor compared with 59.1% (13 out of 22 patients) for those who had a large tumor (**Figure 3**). The difference between these 2 groups was not significant (p=0.82). Distal tumors tended to be greater than proximal lesions, their difference was significant (p=0.0001, Mann-Whitney test). Event-free survival was 70.8% (29 of 41 patients) for proximal located tumors, and 61.5% (24 out of

39 patients) for distally located tumors, however, their short-term event-free survivals were not significant, (p=0.95). The variables that found to predict overall survival in univariate analysis, were the histological response of the primary tumor to preoperative chemotherapy (p=0.0001) and metastasis during or after chemotherapy (p=0.00001). Combining these 2 factors in a logistic regression model, we developed a table for the chance of surviving or death. We did not use cox model for multivariable analysis as the risk of death is not constant during the study, in this model tissue necrosis grade 3 was a significant predictor of overall survival (**Figure 4**).

Other factors used in univariate analysis were aged at admission, location of tumor at tibia comparing to the other sites, proximal tumor site, however, none of them had significant effect on short-term event-free survival also due to the 2 metastasis at presentation, it was not possible to study the effect of these variables on survival.

Factors in relation with tissue necrosis and systemic or local relapse. Age at admission, gender, tumor size, tibia location of tumor versus other areas, and time interval until operation did not have stochastic significance on tissue necrosis or systemic relapse in all univariate analyses (p>0.05, Chi squared test). Patients with more than 90% tissue necrosis had less systemic recurrences that were significant (p=0.0001, Chi squared test)

Discussion. Operative treatment for the local control of osteosarcoma provides an opportunity to examine the histological response of the primary tumor to the preoperative chemotherapy, and the relationship between the histological response and oncological result. As spontaneous necrosis is slight in untreated tumors of osteosarcoma, rarely exceeding 25% in osteosarcoma,[19] it should not affect the measurement of chemotherapy induced tumor necrosis. In patients who have osteosarcoma of the extremities, the extent of tumor necrosis, as determined histologically, is the most important prognostic factor in the prediction of systemic disease after intravenous chemotherapy.[12,18,20-25] Patients who had completed (grade 4) necrosis, or only scattered foci of possible tumor cells (grade 3 necrosis) were considered to have a good response to chemotherapy at 3 years, and the rate of event-free survival for these patients was superior to that for patients who had (grade one) or (grade 2) necrosis. Other variables with possible prognostic impact include tumor size,[26] gender,[27] older age,[27] younger age,[8,28] proximal tumor site,[6,13] high alkaline phosphatase,[10,29] and high lactate dehydrogenase values,[10,30] and location of tumor at tibia. In our study, patients without metastasis during or after therapy or with tissue necrosis more than 90% had longer survival and less death during short-term follow up (40 months) and in combined form of these variables, grade 3 tissue necrosis predicts less death and longer survival, and so a good response to chemotherapy (less than 10% possible tumor) is an important prognostic factor for short-term survival in comparing with systemic recurrence, during or after therapy. Like other issues, tissue necrosis predicts systemic disease well after chemotherapy. We did not find any significant effect for other variables such as gender, age, proximal tumor site, and the location of tumor at tibia in comparing with other sites, on systemic recurrence. Patients with greater degrees of post chemotherapy tissue necrosis have reduced chance of having metastases, so this relationship between histologic response and prognosis suggests that the effect of chemotherapy on the primary tumor correlates with the effect on the micrometastatic disease. Different approaches in treatment of patients after poor response to chemotherapy[12,31-34] by using another chemotherapy regimen after surgery, have usually failed.[33-34] Thus, it is important if there are factors that influence the histologic response to preoperative chemotherapy or to know what combination of drugs is most effective. Unfortunately, in treatment of osteosarcoma, there have been few studies investigating factors that could influence the histologic response of a primary tumor to chemotherapy.[35,36] In a previous study of only 247 patients preoperatively treated with a 3 drug regimen (methotrexate, cisplatin, and doxorubicin), the grade of histologic response to chemotherapy was related to histologic response of the tumor to the methotrexate serum peak at the end of infusion.[36] As we had chosen 3 cycles of preoperative chemotherapy protocols for nearly all of the patients, and our patients' histological subtypes of the tumors were not identified, so it was not possible for us to look for the effect of these 2 factors on tissue necrosis. The effect of other possible variables on tissue necrosis age, gender, and site of primary tumor was searched in this study, although none of them had a significant effect on post chemotherapy tissue necrosis. Although we did not find a significant relationship between tumor size and tissue necrosis, we cannot discuss against the hypothesis of Goldie and Coldman which explains that larger tumors are associated with a higher risk of an inadequate response to chemotherapy due to the presence or development of drug resistant clones.[37] Larger tumors are expected to have more micro metastasis and systemic relapse, however, we did not find significant relationship between tumor size and metastasis during, or after therapy. As our preoperative chemotherapy protocol was the same for all patients and no other factors such as age, gender,

and so forth in this study we found to have significant effect on postoperative chemotherapy, so it seems that the degree of necrosis at the time of surgery is reflected by inherent properties of some tumors that make them intrinsically less responsive to chemotherapy. The property of tumor itself, or the effectiveness of the kind of combined chemotherapy drugs should be considered in the neoadjuvant treatment.

In conclusion, the demographic parameter of patients at presentation seems to play an important role in prognosis , however, the more important factor in prediction of prognosis is available during the therapeutic plan, especially when tissue response to chemotherapy is determined. Although in our study the length of follow up was short yet it is notable to say that there was not such an even short study about osteosarcoma of extremities, and its demographic variables in Iran. This study will be continued to more than 10 years of follow up, thus, more information will be available in the future.

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