Prognostic Significance of Serum Matrix Metalloproteinase 9 (MMP-9) Levels in Patients with Sarcomas

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ABSTRACT

Sarcomas are heterogeneous groups of tumors that are seen rarely. Although great efforts have been made to identify prognostic factors apart from grade, histology and tumor size, they're not so obvious yet. In this study, the prognostic role of serum matrix metalloproteinase (MMP)-9 levels in patients with sarcoma was evaluated.

Eighty-eight patients with a diagnosis of sarcoma were included in the study. Additionally in 14 patients with osteosarcoma, serum MMP-9 levels were analyzed twice, one in preoperative period during neoadjuvant chemotherapy and one in postoperative period. Sixteen healthy volunteers composed the control group. Serum MMP-9 levels have been evaluated quantitively by ELISA method.

Serum MMP-9 levels were higher significantly compared to control group ($p<0.005$). Patients with metastatic disease have higher serum MMP-9 levels compared to the non-metastatic ones which were statistically non-significant (p: 0.9). Similarly, no difference in serum MMP-9 levels was observed between patients with intermediate-high grade tumors and with low grade tumors. Moreover, statistically significant decrease in serum MMP-9 levels was observed in patients in postoperative period compared to preoperative period (p: 0.016).

Although this study was performed in small number and heterogeneous group of patients, the difference in serum MMP-9 levels between sarcoma patients and control group, especially in the presence of measurable lesions and in postoperative period of the same patients, suggests that serum MMP-9 levels may be used as a tumor marker in patients with sarcoma.

Key Words: Serum MMP-9, Sarcoma

ÖZET

Sarkomlu Hastalarda Serum Matriks Metalloproteinaz-9 Düzeyinin Prognostik Değeri

Sarkomlar oldukça nadir olarak gözlenen heterojen bir tümör grubudur. Tüm çabalara karşın tümör histolojisi, greydi ve tumor boyutu dışında bir prognostik faktör tayin edilememiştir. Bu çalışmada sarkomlu hastalarda serum matriks metalloproteinaz-9 (MMP-9) düzeyinin prognostik değeri araştırıldı.

Çalışmaya histopatolojik olarak sarkom tanısı konulmuş olan 88 hasta alındı. Osteosarkom tanılı 14 hastada serum MMP-9 düzeyi operasyon öncesinde neoadjuvant kemoterapi döneminde 1 kez ve postoperative dönemde 1 kez olmak üzere iki kez örneklendi. Onaltı sağlıklı gönüllü kontrol grubunu oluşturdu. Serum MMP-9 düzeyleri ELISA yöntemi ile ölçüldü.

Serum MMP-9 düzeyleri sarkomlu hastalarda kontrol grubuna göre anlamlı oranda yüksek bulundu ($p<0.005$). Metastatik hastalık varlığında istatiksel öneme ulaşmayan bir yükseklik saptandı (p: 0.9). Benzer şekildeorta-yüksek greydli tümörü olanlarla düşük greydli tümörü olan hastalar arasında serum MMP-9 düzeyleri arasında anlamlı bir farklılık gözlenmedi. En önemlisi, serum MMP-9 düzeyleri açısından postoperatif dönemde yapılan örneklemelerde peraoperatif sürece göre anlamlı oranda azalma saptandı (p: 0.016).

Her ne kadar bu çalışma sınırlı sayıda ve heterojen bir grup hastayı içerse de sarkomlu hastalar ile kontrol grubu arasında, özellikle aynı hastaların preoperatif ve postoperatif dönem serum MMP-9 düzeyleri arasındaki farklılık serum MMP-9 düzeylerinin sarkomlu hastaların takibinde faydalı olabilecek bir belirteç olabileceğini düşündürmektedir.

Anahtar Kelimeler: Serum MMP-9, Sarkom

INTRODUCTION

Sarcomas are rarely seen tumors, and constitutes 1% of all malignancies. They are mesencyhmal in origin and biological behaviors are completely different from epithelial tumors. Due to heterogeneity in terms of both localization and histological properties, determination of prognostic factors in sarcomas is difficult unlike to other epithelial tumors. Today, grade of the sarcoma is the most important prognostic factor. However, rarity and complex histological patterns may cause some troubles in determination of grade even by experienced pathologists. Moreover, grade of tumor may be evaluated differently by different pathologists; in other terms, grade evaluation may be subjective sometimes. New prognostic markers are warranted in this era.

Matrix metalloproteinases (MMP) are responsible from the degradation of extracelluar matrix. In addition to physiological functions in inflammation, wound healing and ovulation, they also have a role in some pathological process including periodontitis, rheumatoid arthritis, osteoarthritis and bulluos lesions of skin serum MMP levels (1). Cancer is the one of the major cause of pathologically increased MMP levels. MMP-2 and MMP-9 are the major enzymes responsible from degradation of type IV collagen which is the major component of basement membrane in phase of cancer invasion. In vitro studies showed the relationship between serum MMP levels and both tumor invasion and prognosis (2). Especially in breast, colorectal, bladder and ovarian cancers, serum MMP-9 levels have prognostic significance (3-6). Unlikely to other malignancies, there are few studies in patients with sarcomas (7,8). To date, the role of MMP-9 in patients with sarcoma has remained unclear. Here in, we reported the prognostic role of serum MMP-9 levels in patients with sarcoma.

PATIENTS AND METHODS

Patients

Eighty-eight patients with histopathological diagnosis of sarcoma were taken into the study. All patients were required to have adequate renal, hepatic and bone marrow functions. They were in good performance status (performance status ≤ 2). Control group composed of 16 healthy volunteers. Peripheral blood samples were taken from both patient and control group; additionally, in 14 patients blood samples were taken in both preoperative period and also in postoperative period. Local ethical committee approved the research protocol.

Method for serum MMP-9 levels

Blood samples from the patients were taken in the morning 1 to 2 weeks before surgery or chemotherapy. Blood samples from the patients and controls were into dry tubes and sera separated from cellular elements by centrifugation within half an hour after blood sampling. The sera were stored at -80°C until assayed. Serum MMP-9 level was determined by ELISA (Oncogene Research Product, MMP-9 ELISA cat ≠ QIA56).

Statistical Analysis

Statistical analyses were done by SPSS 11(SPSS Inc., IL, and USA) statistical software. Serum MMP-9 levels in both patient and control group was analyzed with student t-test. The relation with serum MMP-9 levels and patient characteristics' were evaluated by Kruskal-Wallis test.

RESULTS

A total of 88 patients were evaluated. Median age was 35 (12-78) and 62 (70.4%) of them were under 50 years. Forty-eight of patients (54.5%) were male and 40 (45.5%) were female. In control group, median age was 32 (32-65) and 7 (43.7%) of them were male. Characteristics of the patients were summarized in Table 1.

Histopathologically, 28 (31.8%) patients were osteosarcoma, 11 (13.6%) were malignant mesencyhmal tumor, 9 (10.2%) were Ewing Sarcoma, 8 (9.1%) were Liposarcoma, 7 (7.95%) were Malignant Fibrous Histiocytoma and Leiomyosarcoma. In 45 (51.2%) patients, metastatic disease was established. Of them, 15 (33%) were metastatic at initial presentation. The most common metastatic sites were lung in 26 (58%) patients and liver in 5 (12%) patients. Sixty four (72.8%) patients had intermediate-high grade tumor, while 9 (10.2%) patients had low grade tumor. However, in 15 (17%) patients no grade determination could be done. In 14 patients with osteosarcoma, serum MMP-9 levels were evaluated both preoperative and postoperative period.

In sarcoma group, serum MMP-9 levels were significantly higher than control group (mean: 301.2 ± 15.2 ng/ml vs. 187.7 ± 14.7 ng/ml, $p<0.005$). Serum MMP-9 levels of patients were summarized in Table 2. In patients with metastatic disease serum MMP-9 levels insignificantly higher compared to non-metastatic patients (mean: 304.1 ± 20.9 ng/ml vs. 300.9 ± 20.9 ng/ml, $p=0.9$). Similarly, in respect to grade of the tumors, no correlation was detected. In patients with intermediate-high grade tumors serum MMP-9 levels were 274.8 ± 16.9 while patients with low grade tumors had serum MMP-9 levels of mean 335.1 ± 30.4 ng/ml ($p= 0.2$). In 14 patients with osteosarcoma who were taking neoadjuvant chemotherapy, serum MMP-9 levels significantly decreased after the operation compared to

Table 1. Characteristics of the patients

Characteristics	Patient number (%)
Age (Median)	35 (range, 12-78)
≤50 years	62 (70.4)
>51 years	26 (29.5)
Sex	
Male	48 (54.5)
Female	40 (45.5)
Presence of Metastatic Disease	
Yes	45 (51.2)
No	43 (48.8)
Metastatic site	
Lung	26 (58)
Liver	5 (12)
Multiple localizations	14 (30)
Grade	
Low grade	9(10.2)
Intermediate-high grade	64 (72.8)
Unknown	15 (17)
Histopathological subgroups	
Osteosarcoma	28 (31.8)
Malignant Mesenchymal Tumors	11 (13.6)
Ewing Sarcoma	9 (10.2)
Liposarcoma	8 (9.1)
Malignant Fibrous Histiocytoma	7 (7.95)
Leiomyosarcoma	7 (7.95)
Rhabdomyosarcoma	6 (6.82)
Synovial Sarcoma	4 (4.54)
Others	8 (8.9)

Table 2. Serum MMP-9 levels

Status of patients	Mean Serum MMP-9 level
All sarcoma patients	301.2 ± 15.2 ng/ml
Healthy controls	187.7 ± 14.7 ng/ml
Metastatic disease presence	
Yes	304.1 ± 20.9 ng/ml
No	300.9 ± 20.9 ng/ml
Grade of tumor	
Low Grade	335.1 ± 30.4 ng/ml
Intermediate-High Grade	274.8 ± 16.9 ng/ml
Patients taking neoadjuvant Chemotherapy	
Before surgery	283.3 ± 40.6 ng/ml
After surgery	181.3 ± 38.1ng/ml

preoperative period (mean: 283.3± 40.6 ng/ml vs. 181.3 ± 38.1 ng/ml, p= 0.016).

DISCUSSION

Sarcomas are rarely seen tumors and contain a heterogeneous group of subtypes. In respect to other solid tumors, only limited numbers of studies to specify the prognostic factors are present in literature. Although grade of the tumor still remains as the major prognostic factor, due to lack of standardization in grading systems, new prognostic markers are needed. MMP's are one of the candidates of "prognostic factor for sarcomas". MMP-9 generally has been positively associated either with creating pathways for tumor cell invasion or generating/releasing bioactive factors such as vascular endothelial growth factor and transforming growth factor-ß enhancing tumor angiogenesis and progression (9,10). In this paper, we reported the role of serum MMP-9 as a prognostic factor in patients with sarcomas.

We detected statistically significant higher serum MMP-9 levels compared to healthy controls. Similarly, Pallota et al. (11) found serum MMP-9 levels were significantly higher in patients with soft tissue sarcomas compared to both healthy controls and benign lesions. However, in another study by Soini et al. (12) the relation between serum MMP-9 levels and sarcomatous lesions were evaluated, and no correlation was observed. Although higher serum MMP-9 levels are expected in patients with metastatic sarcoma, this was not shown in our study. The data from literature is controversial in this issue. Benassi et al. (13) showed that there was a correlation between serum MMP-9 levels and incidence of metastatic disease only in patients with liposarcoma. In another small study, Himmelstein et al. (14) it was found that in 4 of 5 metastatic patients, immunhistochemically MMP-9 stain was strongly positive and it was urged that MMP-9 expression was important in the development of lung metastases. Foukas et al (15) reported that MMP-9 was important by means of prediction of metastases, disease free-survival and overall survival. Moreover, in patients with osteosarcoma, the prognostic significance of serum MMP-1, MMP-3, MMP-9 and tissue inhibitors of MMP (TIMP)-1 were evaluated and only serum MMP-3 levels were important in the prediction of metastases compared with tumor volume and histological grade (16). The differences may be related with the number of included patients. However, as known, metastase biology includes multiple steps although degradation

of basement membrane was the critical stage. Main function of MMP-9 is evolved during only this critical stage and it's obvious that without other steps, by only MMP-9 levels, the malignant potential of tumor could not be determined. In concordance, it was pointed that not only MMP's but also MMP/TIMP balance was more important in metastatic capacity in patients with breast, cervix, colon, prostate, bladder and renal cell tumors. Similiarly, Maguire et al. (17) reported that increased MMP-9/TIMP-1 levels were related with decreased disease free-survival in patients with soft tissue sarcomas. Increased MMP-2 levels with decreased TIMP-2 levels were claimed with metastases in another study (13). However, Uchibori et al (18) reported that in patients with osteosarcoma, MMP-9 levels had no impact neither overall survival nor disease free survival; in contrast, co-overexpression of MT1-MMP and MMP-2 associated with reduced disease free survival.

More strikingly, in 14 patients with osteosarcoma an important decrease in serum MMP-9 levels was observed with the removal of tumor. Similarly, Pallota et al (11) reported that in a small number of patients removal of tumor was associated with statically significant drop in serum MMP-9 levels; however, metastatic disease was gradually observed in patients showing an increase in serum MMP-9 levels in the follow-up.

Nowadays tumor grade was most important prognostic factor in sarcomas. In current study, there was no significant difference in serum MMP-9 levels in terms of tumor grade. In one study, there was correlation between tumoral grade and serum MMP-9 levels only in patients with liposarcoma. However, it is not true to conclude that there was no correlation between tumor grade and serum MMP-9 levels, due to heterogeneity and small number of patient.

In current study, although number of patients was low and heterogeneous, serum MMP-9 levels were significantly higher than healthy controls. Especially the drop in serum MMP-9 levels with the removal of tumor exhibits that serum MMP-9 levels may be helpful in the follow-up of patients. Larger studies are warranted in this era.

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