

## Immunohistochemical Expression of Matrix Metalloproteinase-9 in Jaws and Long Bones Osteosarcomas

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### ABSTRACT:

#### BACKGROUND:

Matrix metalloproteinases (MMPs) are a class of matrix basement membrane degrading enzymes which were shown to be associated with metastases in several human tumors.

#### OBJECTIVE:

This study is aimed to investigate the potential effect of MMP-9 in imparting the frequently reported different behavioral pattern between jaws and long bones osteosarcomas.

#### METHODS:

Tissue blocks of ten cases of jaws osteosarcomas and another ten of long bones osteosarcomas were collected and stained immunohistochemically with monoclonal antibodies to MMP-9.

#### RESULTS:

The majority of cases (70%) were positive for MMP-9 expression which indicates a role in tumor spread, however; there was no significant difference between the sites.

#### CONCLUSION:

The study indicates that MMP-9 is probably not involved in the biologic differences between jaws and long bones osteosarcomas.

**KEYWORDS:** jaw osteosarcoma, matrix metalloproteinase-9

### INTRODUCTION:

Osteosarcoma (OS) is a primary malignant tumor of the skeleton characterized by the direct formation of immature bone or osteoid tissue by the tumor cells<sup>(1)</sup>. It is the most common non-hemopoietic primary tumor of bones<sup>(2)</sup>. Most patients are under 25 years of age at presentation with a smaller proportion after 40 years associated with pre-disposing factors as Paget's disease and irradiation<sup>(3)</sup>. The tumor mostly involves the metaphyseal areas of the long bones, less commonly; it affects other sites including pelvic bones, vertebral column, cranial bones or jaws<sup>(1)</sup>. It is presented clinically as an expansile mass associated with deep pain with a radiographic evidence of new bone formation<sup>(2)</sup>.

Histopathologically, osteosarcomas are subdivided

into three major subtypes, osteoblastic, chondroblastic and fibroblastic according to the predominant histologic component (1-3). Osteosarcomas of the jaws are uncommon, representing less than 10% of all OS. They have been diagnosed in patients ranging from young children to the older adults; however, the mean age for patients with OS of the jaws is about a decade older than the mean age for OS of the long bones<sup>(4,7)</sup>. A predominance of the chondroblastic histologic subtype is evident in jaw tumors in contrast to the osteoblastic subtype predominance in other locations<sup>(1,2,6)</sup>. Many past and present investigators believe that OS of the jaws are less aggressive than those occurring in the long bones<sup>(5)</sup>; where OS of the jaws, often show little cellular atypia and late metastasis<sup>(4,6)</sup>.

Matrix metalloproteinases (MMP) are a class of matrix and basement-membrane-degrading enzymes which are important for matrix turnover. MMP-9 is

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important for neoangiogenesis both in normal tissues and tumors, and it is well known that tumors stimulate the growth of new blood vessels. Once these vessels penetrate a microscopic primary neoplasm the lesion acquires the potential to grow and become more aggressive, potentially threatening life. The production of MMP-9 by tumor cells is associated with the development of metastases in laboratory rodents <sup>(8)</sup>. Metastatic lesions of osteosarcoma in children have been shown to be strongly positive for MMP-9 <sup>(9)</sup>. This article is an attempt to clear whether there is a difference in MMP-9 expression between jaw and long bones osteosarcoma that could lie behind the different biological behavior.

### **MATERIALS AND METHODS:**

#### **Sample**

Ten formalin-fixed paraffin-embedded tissue blocks of OS of the jaws, and another 10 of the long bones were retrospectively and selectively collected from the Department of Oral Diagnosis / College of Dentistry / Baghdad University and the Baghdad hospital of surgical specialties for the period from 1986 to 2005. Four-micrometer-thick sections were cut from each paraffin tissue block and stained with hematoxylin and eosin for diagnostic confirmation and histological subtype recognition. Tumors were classified into high and low grades sarcomas according to the degree of cellularity, atypia and extracellular matrix production (10). Another 4-um section was cut from each tissue block and mounted on positively charged slides (Fisher Scientific, Philadelphia, PA, USA) to be stained with monoclonal antibodies to MMP-9 (USBiological-M2425-01L).

#### **Immunohistochemical staining procedure**

Slides were baked in hot air oven at 65\_C overnight. Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps.

Endogenous peroxidase activity was blocked with 3% hydrogen peroxide followed by blocking the nonspecific antibody binding with normal goat serum (USBiological-I7506A); this was followed by the application of the primary antibodies with a dilution of 1:50. The slides were incubated for 1 h at 37\_C and then kept at 4\_C in a humid chamber overnight. Next day, after washing with PBS, biotinylated antimouse IgG (USBiological-I7506B) were applied to the sections, incubated and rinsed with a stream of PBS. Conjugated antibodies were visualized with DAB chromogen. Sections were counterstained with Mayer's hematoxylin for 1–2 min, dehydrated and mounted.

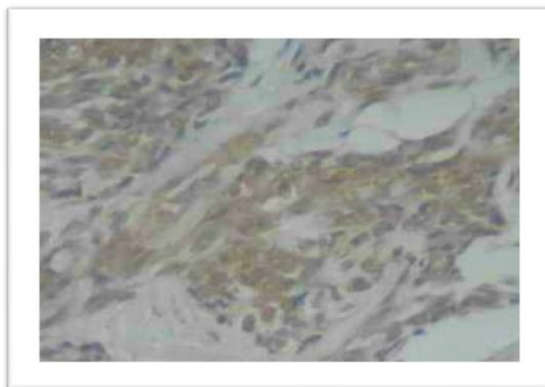
#### **Assessment of immunohistochemical results**

In each tissue section, at least five representative fields were selected to be evaluated with a 40X objective. Counting of positive cells within each field was carried out and divided by the total number of cells per that field to obtain the percentage of positive tumor cells. An average of these multiple readings was obtained where any reading below 10% was considered negative, and it was positive otherwise (11).

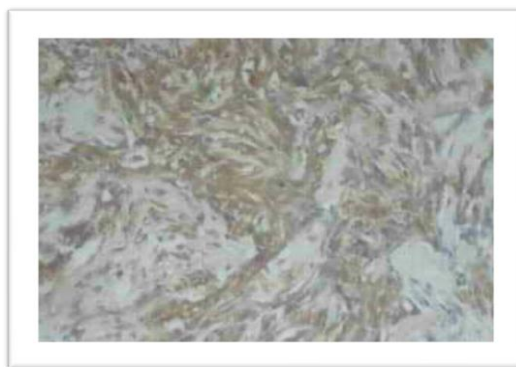
### **RESULTS:**

The study showed an immunohistochemical expression for MMP-9 (Figure 1,2 and 3) in 70% of both sites with no significant difference. Moreover, no relationship was found between MMP-9 expression and age, sex, grade or histological subtype.

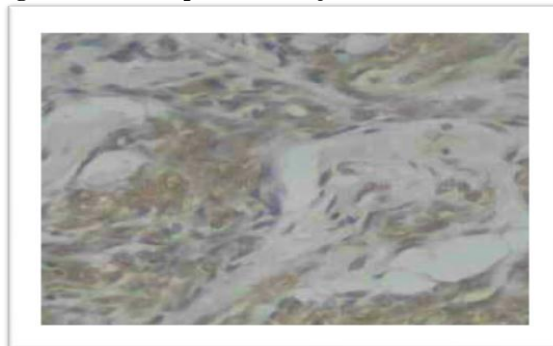
With the exception of age which was significantly higher in jaw cases, comparing other parameters as sex, histological subtypes and grades showed no significant difference between sites (Table 1).



**Figure 1: MMP expression in a jaw osteosarcoma (X400)**



**Figure 2: MMP expression in a jaw osteosarcoma (X100)**



**Figure 3: MMP expression in a long bone osteosarcoma (X400)**

**Table 1: Clinical, histopathological and immunohistochemical comparasion**

		Jaws osteosarcomas	Longbones osteosarcomas	Test	P value
Age (Mean±SD)		28.7± 15.4132	17.4±4.325634	T test	0.037*
MMP positive		7	7	Chi square	1 <sup>NS</sup>
MMP negative		3	3		
Grades	H	3	2	Chi square	0.596 <sup>NS</sup>
	L	7	8		
Sex	M	4	6	Chi square	0.371 <sup>NS</sup>
	F	6	4		
Histological subtype	Osteoblastic	4	6	Chi square	0.371 <sup>NS</sup>
	Chondroblastic	6	4		

\* Significant difference

<sup>NS</sup> Non-significant difference

**DISCUSSION:**

The matrix metalloproteinases (MMPs) constitute a family of over 24 members, which collectively are capable of degrading virtually the entire ECM. Several studies have documented the importance of MMP-mediated ECM destruction in the successful dissemination of several tumor types. MMPs are implicated in a wide variety of roles that can assist tumor initiation, growth, migration, angiogenesis, the selection of apoptosis-resistant subpopulations, and in invasion and metastasis<sup>(8)</sup>. MMP-9, in particular can induce the release of VEGF from basement membrane<sup>(9)</sup> and induce angiogenesis. In a previous study, osteosarcomas of jaws and long bones were compared immunohistochemically regarding proliferation, apoptosis and angiogenic stimulation and found an increased expression of proliferative and angiogenic markers (namely VEGF) in favor of long bones tumors<sup>(12)</sup>. Therefore, this study was performed to investigate the possibility of a role to MMP-9 in the different behavioral patterns expressed in jaws osteosarcomas in contrast to the long bones counterparts.

In an immunohistochemical study, Yoo et al. (2005) found that MMP-9 is expressed in osteosarcomas. Similarly, MMP-9 expression was found in this study which indicates a role for MMP-9 in tumor progression, however; there were an equal number of positive cases in both sites. Despite the small sample size, this result indicate that different behavior could not be attributed to MMP-9 role in extracellular matrix degradation as a mechanism for metastatic enhancement, moreover; the different VEGF expression found between the sites in our previous study was probably the result of a factor other than MMP-9, this result was to some extent in line with the results of Yoo et al. (2005) that demonstrated the

absence of a relationship between MMP-9 expression and microvessel density in osteosarcomas.

In regard to the histological grades, the lack of a relationship with MMP-9 expression came in agreement of a previous study that did not found an immunohistochemical reflection of tumor grades<sup>(12)</sup> which accentuate the subjectivity of the grading system. However; more detailed studies are needed in order to validate or refute this concept.

Many previous studies reported a difference in the age groups affected with jaw osteosarcomas being about a decade older<sup>(4,6,7)</sup>, a similar result was found in this study with a significant difference at the age distribution despite the small sample size.

**CONCLUSION:**

Matrixmetalloproteinase-9 role in osteosarcoma tumorigenesis could be ruled out as a cause to the behavioral difference between jaws and long bones osteosarcomas.

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