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Matrix Metalloproteinase-9 Expression in Dentigerou scyst, Odontogenic keratocyst and Ameloblastoma

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Abstract: Odontogenic lesions have different and controversial behavior from some lesions in other sites of body. These controversies insist the need of further studies about the nature and behavior of these lesions. The aim of the present study was to investigate the stromal and epithelial expression of matrix metalloproteinase-9 in different odontogenic lesions.In this study 88 odontogenic lesions consist of 20 unicystic ameloblastoma (UA), 18 solid ameloblastoma (SA), 20 odontogenic keratocysts (OKC), 20 dentigerous cysts and 10 dental follicle were reviewed by immunohistochemistry for MMP-9 staining.Regarding the paranchymal and epithelial expression of MMP-9, there was a predominance of score 2 in SAs, UAs and OKCs. (Respectively 66.7%, 55% and 45%). But in DCs score 0 was predominant. Statistical analysis revealed significant difference only between ameloblastoma (SA & UA) and DCs. (respectively P= 0.001 & P= 0.002), Stromal expression of MMP-9 in the lesions studied showed a relatively similar pattern. Score 2 was predominant in SAs and OKCs (respectively 72.2% & 45%). In contrast score 1 was obvious in UAs and DCs (respectively 55% and 75%). The results of this study propose that high expression rate of MMP-9 might be one of the reasons for aggressive behavior of ameloblastoma and high recurrence rate of OKC and reinforce the classification of OKC as an odontogenic tumor.

Key words: MMP-9 · OKC · Ameloblastoma · Dentigerous Cyst

INTRODUCTION

Different odontogenic lesions originate from remnant of dental lamina. The potential for further epithelial proliferation in various lesions is different and thus lead to variation in biological behaviors, due to an unknown mechanism [1]. Dentigerous cyst is the most common developmental odontogenic cyst that shows an indolent behavior and its recurrence is rare after removal [2]. The odontogenic keratocyst (OKC), recently reclassified by the WHO (World Health Organization) as a keratocystic odontogenic tumor, is a developmental cyst with entirely distinct behavior from other odontogenic cyst for its aggressive growth and tendency to recur after surgical treatment [3]. Ameloblastoma is a benign odontogenic tumor which is locally aggressive and has a marked invasion potential that result in multiple recurrences after enucleation and curettage [4].

In regard to determine the growth mechanism of odontogenic lesions, as well as the invasion and destructive potential of them, a growing number of studies have tried to identify epithelial and mesenchymal factors. Extracellular matrix destruction is critical for development and dissemination of tumors and this destruction has a role in determining tumor prognosis and in selecting appropriate treatment [5].

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The matrix metalloproteinases are a group of endopeptidases that involved in degradations of extracellular matrix during remodeling in physiologic and pathologic conditions such as, wound healing, growth, inflammation and cancer progression [6-8]. MMPs are classified into different groups, consists of collagenases, gelatinases, matrilysins, stromelysins and membrane type metalloproteinases [9]. Matrix metalloproteinase- 9 (MMP–9) also termed gelatinase B is a 92-KDa protease that specifically destructs collagen type IV, which is the basic structural component of basement membranes [8]. Some investigations suggest that enzymatic destruction of the bone matrix and basement membrane by MMP-9 is involved in the expansion of odontogenic cysts [10-12].

In view of the distinct clinical behavior of OKCs, DCs and ameloblastomas, the objective of the present study was to investigate the immunohistochemical expression of MMP-9 in these lesions.

MATERIALS AND METHODS

Materials: This retrospective study was performed using 88 formalin-fixed, paraffin embedded tissue blocks of odontogenic lesion (20 unicystic ameloblastoma (UA), 18 solid ameloblastoma (SA), 20 odontogenic keratocysts (OKC), 20 dentigerous cysts (DC) and dental follicle (10) which were collected from the Department of Oral and Maxillofacial Pathology, School of Dentistry, Shiraz University of Medical Sciences.

H & E slides of available blocks were reviewed and then cases with definite diagnosis and adequate tissue were selected for immunohistochemical staining (IHC). Cases with severe inflammation were excluded from study.

IHC Staining and Analysis: IHC staining was performed by using Envsion Labled Peroxides System (DAKO, Carpentaria, CA, USA). All the samples have been fixed in 10% buffered formalin and have been embedded in paraffin. Sections with 4µ thickness were prepared, deparaffinized in xylene,rehydrated in graded alcohol and were washed with distilled water. Antigen retrieval was performed by using DAKOcytomation target retrieval solution with PH = 9, for 20 minutes. Internal peroxidase activity was inhibited by 3% H2O2.

Tissue sections were then incubated for 30 minutes with the anti-MMP-9 antibody (Santa Cruz Biotechnology Inc., Sc-19993) at a 1/50 dilution.

Omission of primary antibody was employed as negative control, while tissue of squamous cell carcinoma was used as positive control. Brown cytoplasmic staining was considered as positive.

MMP-9 staining was evaluated according to Gong *et al*., (2009) with some modifications. Ten histologic fields were selected in the epithelial component and in the connective tissue capsule. Immunoexpression of MMP-9 was scored in each case as 0 (<10% immunostained cells), 1 (10%-50% immunostained cells), or 2 (>50% immunostained cells) [13].

Statistical Analysis: Mann-Whitney test was used to compare results. The level of significance was set at 0.05.

RESULTS

In this study MMP-9 immunostaining was evident in all groups of the specimen except dental follicles. The immunoreactivity for MMP-9 was evident both in paranchyma and stroma of ameloblastomas and epithelium and stroma of the cysts. MMP-9 reactivity in the paranchyma of ameloblastoma was differ and mostly located in the cytoplasm of columnar ameloblast like cells and stellate reticulum like cells (Figure 1). MMP-9 expression was also seen in keratinizing cells in acanthomatous ameloblastoma. (Figure 2).

Regarding the paranchymal and epithelial expression of MMP-9, there was a predominance of score 2 in SAs, UAs and OKCs. (respectively 66.7%, 55% and 45%), but in DCs score 0 was predominant (Figure 3-5) (Table 1).

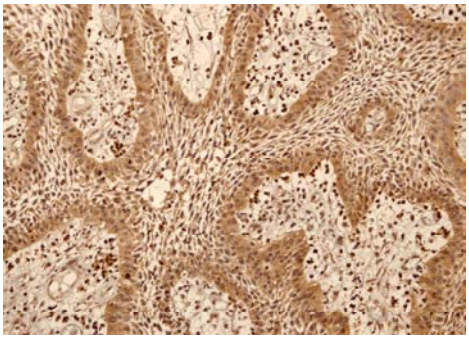

Fig. 1: Paranchymal and stromal staining in ameloblastoma (×200)

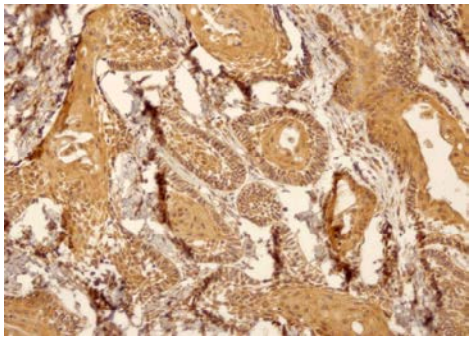

Fig. 2: MMP-9 expression in keratinizing cells in acanthomatous ameloblastoma (×200).

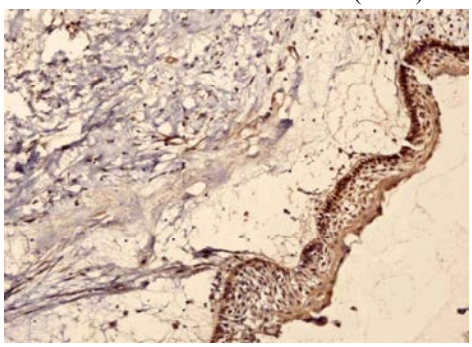

Fig. 3: Paranchymal and stromal staining in unicystic ameloblastoma (×200)

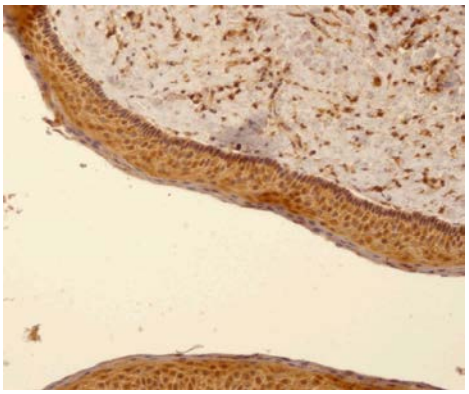

Fig. 4: Epithelial and stromal staining in odontogenic keratocyst (×200)

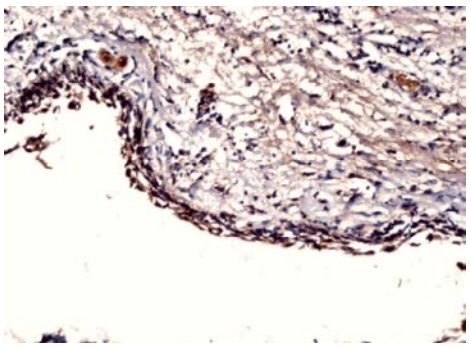

Fig. 5: Epithelial staining in dentigerous cyst (×200)

Table 1: MMP-9 expression in parenchyma of odontogenic lesions and dental follicles.

	Score of paranchymal MMP-9 expression			
lesion	0	1	2	total
SA	0%	33.3%	66.7%	100%
UA	0%	45%	55%	100%
DC	45%	35%	20%	100%
OKC	20%	35%	45%	100%
Dental follicle	100%	0%	0%	100%
Total	26.1%	33%	40.9%	100%

Table 2: MMP-9 expression in stroma of odontogenic lesions and dental follicles

	Score of stromal MMP-9 expression			
lesion	0	1	2	total
SA	0%	27.8%	72.2%	100%
UA	0%	55%	45%	100%
DC	10%	75%	15%	100%
OKC	20%	35%	45%	100%
Dental follicle	100%	0%	0%	100%
Total	18.2%	43.2%	38.6%	100%

Statistical analysis revealed significant difference only between ameloblastoma (SA & UA) and DCs. (respectively P= 0.001 & P= 0.002). Stromal expression of MMP-9 in the lesions studied showed a relatively similar pattern. Score 2 was predominant in SAs and OKCs (respectively 72.2% & 45%). In contrast score 1 was obvious in UAs and DCs (respectively 55% and 75%) (Table 2). Statistical analysis showed a significant differece between DCs and all other groups.

Different cells in the stroma consist of fibroblasts, endothelial cells and inflammatory cells showed MMP-9 immunostaining.

DISCUSSION

Ameloblastoma is the most common and slow growing odontogenic neoplasm which is locally aggressive and shows a high recurrence rate especially if not completely removed [14, 15]. Between odontogenic cysts, OKC shows aggressive behavior with higher rates of recurrence than other types of odontogenic cysts, as well as a tendency to invade adjacent tissue which was comparable to ameloblastoma [1], So the term Keratocystic odontogenic tumor (KOT) was suggested by WHO, due to its aggressive nature [16].

Most of investigators related the distinctive behavior of KOT to the features of its epithelial lining [17-19]. But Browne (1975) who was first to propose that connective tissue wall also has a role in the pathogenesis of OKC [20].

Regulation of cell functions, such as differentiation, apoptosis, proliferation and migration is controlled by epithelial – mesenchymal intraction [21, 22]. The extracellular matrix (ECM) is a dynamic structure which has an important role in both normal and pathologic process such as inflammation, angiogenesis, wound healing and invasion of tumor [23].

MMPs are important proteases that can lead to structural and functional modification of ECM component. Their expression was poor in tissue under physiologic condition, but their expression was increased in pathologic conditions due to the activity of MMPs and their inhibitors [24]. Considering the odontogenic lesions, the expression of MMPs was observed in ameloblastoma, dentinogenic ghost cell tumor, OKC, DC and radicular cyst [12, 13, 24].

In this study MMP-9 reactivity was not seen in dental follicle which was supported the previous findings that MMPs are poorly expressed under physiologic conditions. In the present study MMP-9 immunoreactivity was higher in ameloblastomas and OKCs than dentigerous cysts. This difference in MMP-9 expression might explain the variable behavior of these lesions and highly aggressive and invasive behavior of ameloblastomas and OKCs and supported the notion that OKC having a neoplastic nature. Expression of MMP-9 in epithelial cells, contribute to the more aggressive behavior of these lesions through degradation of basement membrane [2]. Kubota *et al*, 2000, conducted that the active form of MMP-9 was present in the fluids of 75% of OKCs and in only 30% of DCs and radicular cyst [3]. Santos *et al*, 2011, also demonstrated higher expression of MMP-9 in OKCs than DCs [2].

In accordance with these finding, in the present study MMP-9 immunoexpression was higher in epithelial cells of OKCs compared with DCs ($P > 0.05$).

In the previous study, MMP-9 expression in ameloblastoma was found only in the cells of the periphery of the nests [15, 25] but in the present study sypported by Ribeiro *et al*, [24] and Florescu *et al*, [26], MMP-9 expression was found in both the central portion and peripheral cells of the nests.MMP-9 expression in ameloblastomas is possibly related to cell differentiation that occurs in tumor cells. Peripheral cells of the nests, are high columnar or cuboidal cells resemble the ameloblasts, but also, did not reach the maturity to form enamel [27].

Regarding the MMP-9 expression in the stroma and fibrous capsule of the studied odontogenic lesions, higher expression of this protein was seen in SAs and OKCs compared with UAs and DCs. This finding was in agreement with Silverira *et al*, [12] and Santos *et al*. [2] who showed higher expression of MMP-9 in OKCs compared with DCs. Kumamoto *et al*, detected strong MMP-9 reactivity in stroma of ameloblastoma, suggesting that over expression of this protein by neoplastic cells lead to the neoplastic transformation of odontogenic lesion and aggressive behavior of these tumors [25].

Many investigators suggested that the MMPs present in the ameloblastoma cause bone resorption, that lead to release of cytokines and growth factors entrapped within the matrix of bone [28-31]. Taken together these findings and the results of the present study suggest that higher expression of MMP-9 in the mesenchymal cells, confirms the participation of the enzymes in the degradation of ECM components and promoting lesion growth.

It should be emphasized that through immunohistochemical technique, in this study as well as in the one from Kumamoto *et al*. [25], it is possible to detect the presence or absence of MMP-1, MMP-2 and MMP-9 in the tumors tested, though it is not possible to determine the enzymatic activity of these metalloproteinases. But Ikebe *et al*. [32] observed that there was a correlation between the results of the zimography technique performed to verify the activity of metalloproteinases and the degree of expression displayed by immunohistochemical tests.

CONCLUSION

The results of this study propose that high expression rate of MMP-9 might be one of the reasons for aggressive behavior of ameloblastoma and high recurrence rate of OKC and reinforce the classification of OKC as an odontogenic tumor.

We have also showed that connective tissue cells are as important as epithelial cells in the biological behavior of these lesions.

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