Original Article

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Dentistry Section

Common Matrix Metalloproteinases (MMP-8, -9, -25, and -26) Cannot Explain Dentigerous Cyst Expansion

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ABSTRACT

Objective: Mechanisms of the dentigerous cyst formation from the normal eruption follicle is unknown but disturbances in the proteolytic activity have been suspected, since the growth of these cysts is accompanied by local bone destruction. The aim of the present study was to evaluate the expression of matrix metalloproteinases (MMP) in human dental dentigerous cysts and healthy dental follicles.

Materials and Methods: We studied 10 patients with dentigerous cysts and 10 healthy dental follicles from the lower jaw in respect to their immunoexpression of MMPs -8, -9, -25, and -26 and tissue inhibitor of metalloproteinases -1 (TIMP-1).

Results: MMP-8 was expressed slightly more in cyst epithelium than in odontogenic epithelium of healthy controls dental follicle but the difference lacked statistical difference. Other MMPs and TIMP-1 did not differ regarding the studied specimens.

Conclusion: Differences in MMP expression cannot solely explain the cyst expansion suggesting the potential involvement of other osteolytic mechanisms.

Keywords: Dental follicle, Dentigerous cyst, Matrix metalloproteinase, Mmp, Timp-1, Third molars, Wisdom tooth

INTRODUCTION

Dentigerous cyst (DC, Follicular cyst) is the most common developmental odontogenic cyst found around unerupted teeth originating from dental follicle. Most often the cysts are detected early and local surgery can adequately solve the clinical problems but sometimes the cyst can grow considerably large and their resection leads to the need of reconstruction and can cause clinical challenges. Also, carcinomas arising in DC has been reported, but is rare [1,2]. Since major bone remodeling and destruction of bone are associated with cyst expansion, protease-related hypothesis is often linked to cyst formation. Matrix metalloproteinase (MMP) are a large family of enzymes which take part in many proteolysis associated pathological conditions [3]. Role of MMPs and their tissue inhibitors has also been associated to several different cysts types found in maxillofacial region, including follicular cysts [4-6]. However, all the mechanisms how the cyst develops and eventually destructs the bone have not been completely clarified.

DCs are found most often in association of mandibular third molars. A healthy dental follicle (HDF) around unerupted third molar tooth is mainly build up of connective tissue with islets of odontogenic epithelium whereas DC has 2-4 cell-layer-thick of non-keratinized stratified squamous or flattened-low cuboidal epithelium surrounding the cyst cavity [7]. Connective tissue capsule of HDF have been earlier considered to be mainly free of inflammatory cells except in the case of pericoronitis. Interestingly some results, however, suggest that the destructive potentiality is also present in the healthy follicles mediated by proinflammatory cytokines and their receptors [8]. This is an interesting finding since the risk/benefit ratio for the prophylactic extraction of un- or partially erupted wisdom tooth is under constant discussion in clinical practice. In this study we focused on DCs epithelium and as its comparison for a HDF containing non-cystic odontogenic epithelium. The objective of the study was to compare the immunoexpression of several matrix metalloproteinases (MMP -8, -9, -25 and -26) and their inhibitor tissue inhibitor of metalloproteinases-1 (TIMP-1), all commonly associated to bone remodeling, in the DC epithelium and their asymptomatic counterparts, HDF.

MATERIALS AND METHODS

Patients and samples: DC (n=10) were collected from the files of Department of Oral Pathology, Institute of Dentistry, University of Helsinki. They were histologically diagnosed as DC by JW. Dental follicles (n=10) were collected at the Department of Oral and Maxillofacial Surgery, Institute of Dentistry, University of Helsinki, from patients having a symptomless unerupted third molar referred to operative extraction for prophylactic or orthodontic reasons. The Ethics Committee of Institute of Dentistry, University of Helsinki, approved the study and all patients gave an individual written permission for the study. These teeth had no signs of inflammation either clinically, nor radiologically or later histologically (evaluated by Jaana Wahlgren) [Table/Fig-1].

Immuhistochemical staining

Immunohistochemistry was done as described elsewhere [3]. Polyclonal antibody for MMP-8 (diluted 1:400 1% BSA/PBS) and polyclonal antibody for MMP-9 (1:1200). Polyclonal antibodies for MMP-25 (diluted 1:1800 1% BSA/PBS), MMP-26 (1:2500) and TIMP-1 (1:250) [9-12]. Specificity of the staining was determined replacing the primary antibody with buffer alone and with buffer-diluted normal rabbit serum (DAKO, Glostrup, Denmark). The immunohistochemical stainings were performed on 4-µm-thick, formalin fixed, paraffin-embedded tissue sections using VECTASTAIN Elite ABC Kit PK 6101 (Vector laboratories, Burlingame, CA, USA) as previously described [13]. After deparaffinization and pepsin incubation (exception TIMP-1 pepsin incubation replaced with Microwave treatment; Dako) followed by washes, the tissue sections were incubated in 0,3 % H_2O_2 in methanol to quench the endogenous peroxidase activity. Non-specific binding was blocked with normal blocking serum (VECTASTAINElite ABC Kit). The sections were incubated overnight with the primary antibody at 4^0C in a humid chamber, followed by washes in PBS, and incubation consecutively with biotinylated secondary antibody solution (antirabbit IgG) and with VECTASTAIN Elite ABC reagent. The tissue sections were stained with 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO, USA), diluted in N,N-dimethylformamide (Merck, Darmstadt, Germany) counterstained with Mayer's hematoxylin, and mounted in DAKO's glycergel (DAKO Corporation, Carpinteria, CA, USA).

Tooth	Sex	Age(y)	PAD/Histological analysis
Dentigerous cysts around wisdom tooth n=10			
48	F	21	Dentigerous cyst
48	M	24	Dentigerous cyst
38	F	62	Dentigerous cyst
38	M	45	Dentigerous cyst
48	F	55	Dentigerous cyst
38	M	52	Dentigerous cyst
38	M	44	Dentigerous cyst
38	M	23	Dentigerous cyst
38	F	36	Dentigerous cyst
38	M	32	Dentigerous cyst
$\overline{X}$ = 39			
Dental follicles around wisdom tooth (unerupted) n=10			
18	M	20	Dental follicle
48	M	24	Dental follicle
48	F	23	Dental follicle
48	F	21	Dental follicle
48	F	23	Dental follicle
38	F	22	Dental follicle
38	F	29	Dental follicle
18	F	23	Dental follicle
28	F	23	Dental follicle
38	M	19	Dental follicle
$\overline{X}$ = 22			

[Table/Fig-1]: Clinical charasteristics of patients with dentigerous cysts and a healthy dental follicle around wisdom teeth

Evaluation of immunostaining

The staining positivity and intensity of different MMPs in DCs and HDF were analysed. Areas of two different microscopic slides of each dental follicle and DCs were inspected with a light microscope using 400x magnification. The epithelial area in all the samples was inspected. Two-step score was used for the positivity of staining (0, no positive staining; 1, positive staining). When staining was observed, the intensity was graded using a three-step scale (1, faint; 2, moderate; 3, strong positive staining). The results of the independently inspected epithelial areas were summarized.

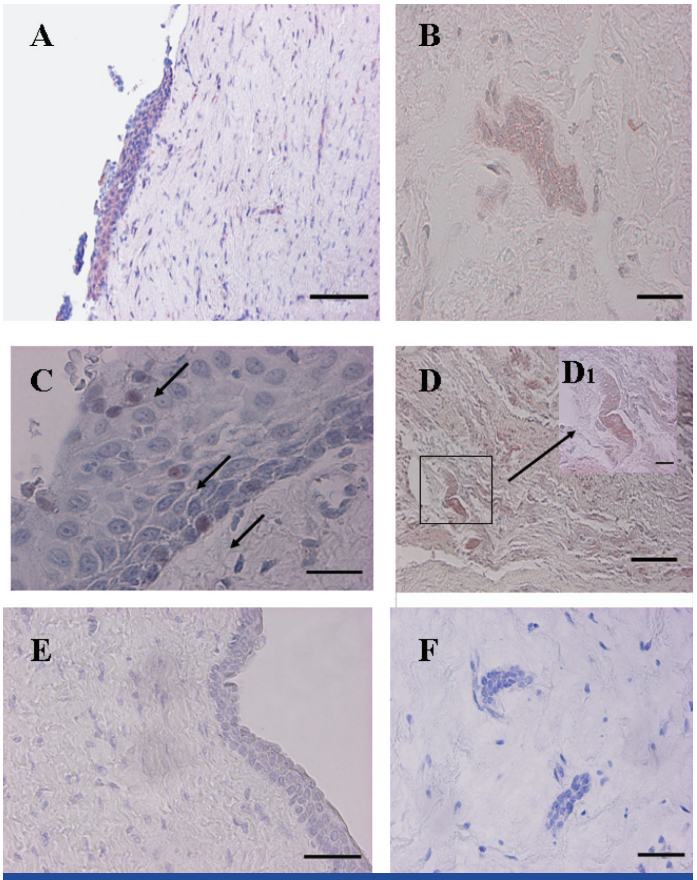
STATISTICAL ANALYSIS

To examine statistical significances in the differences of MMPs in the BM area between different odontogenic cysts, the Fisher's non-parametric exact test in SPSS program was used. To highlight possible clinical significance of strong expression the differences between groups having lower scoring (0-2) and high scoring (3) were also independently analysed.

RESULTS

Among MMPs only MMP-8 was slightly more expressed in DC than in the epithelium of the dental follicles.

The DC epithelium lined the cyst cavity whereas the dental follicle consists mainly of connective tissue and only few epithelial islets of odontogenic epithelium were detected. The immunohistochemical staining revealed expression of MMP-8 in the epithelium of the DCs



[Table/Fig-2]: Expression of MMP-8 and MMP-9 in DC and HDF MMP-8 can be detected by immunostaining in cyst epithelium (A) as well as to some extent in dental follicles (B). There is very mild MMP-9 expression in both DC and HDF (C,D) and D1 represents epithelial remnants in dental follicle. The Normal HE-staining can be seen for cyst epihelium (E) and odontogenic epithelium (F) from dental follicle. The scale bar is 100 µm

[Table/Fig-2a]. Some signal was also seen in polymorphonuclear (PMN) cells and some fibroblast-like cells being present in connective tissue. Milder positive staining for MMP-8 was seen in the odontogenic epithelium islands in HDF´s [Table/Fig-2b]. However, the results are not statistically significant (p = 0.255) and the result remains the same even though only high positive signal (3) is compared to the low positives (0,1,2) (p = 0.155).

Expression of MMP-9, -25, -26 or TIMP-1 does not differ in DC and HDF

Single epithelial cells showed MMP-9 positivity in the DC´s though the positivity was very mild. In the cyst capsule only sparse endothelial cells and some PMN-cells were MMP-9 positive close to the cysts epithelium and in the HDF some epithelial cell islands in connective tissue were found to be positive for MMP-9. In all HDF´s odontogenic epithelium was present and mildly positive for MMP-9 [Table/Fig-2c-f]. There was no statistical difference between these groups (p= 0.370). For MMP-25 immunopositivity was detected in the epithelial keratinocytes and in the basement membrane zone and positivity for MMP-25 antibody was detected also in HDF odontogenic epithelium. In stroma MMP-25 positivity was seen in some mononuclear inflammatory cells and BM zone cells (Not shown). The results between the groups were not statistically significant (p = 0.326). MMP-26 positivity was not seen in the epithelial cells of DC ´s; mild staining was sometimes seen in the basement membrane zone and connective tissue capsule in collagen bundles. In the HDF´s MMP-26 positivity was seen at the stromal connective tissue but not in the epithelial islands (not shown). The results were not statistically significant (p = 0.639). Strong TIMP-1 positivity was identified in both HDF´s and DC´s. In DC´s connective tissue had strong TIMP-1 positivity in fibroblast- and macrophage-like cells. In HDF TIMP-1 was expressed in the

	DC +/n	HDF +/n
IH MMP-8	8/10	5/10
IH MMP-9	6/10	4/10
IH MMP-25	9/10	7/10
IH MMP-26	8/10	9/10
IH TIMP-1	10/10	10/10

[Table/Fig-3]: MMPs in samples of dentigerous cysts and healthy dental follicles IH = immunohistochemistry; DC = dentigerous cyst; HDF = healthy dental follicle; + = epithelial cells positive in the sample; n = number of samples studied

island of odontogenic epithelium (not shown). Also in the case of TIMP-1 there was no difference at all between the two groups (p = 1.000). The overall staining can be detected from [Table/Fig-3].

DISCUSSION

It has been demonstrated that enhanced expression of MMPs is associated with aggressively behaving odontontogenic cysts type, earlier called keratocyst, currently keratocystic odontogenic tumour [14,15]. MMPs are also connected to several other bone destructive conditions [3,6,13,14]. Lately, it has been noted that proliferation and expansion of cyst epithelium is greatly influenced by the inflammatory response [16]. This is an interesting finding since most of the MMPs can be produced by inflammatory cells [17] and chronic inflammation quite often involves also the asymptomatic third molars [18]. The proinflammatory cytokines have also been demonstrated to be able to evoke the bone destructing potential of the HDF's around asymptomatic and clinically innocent looking third molars [8]. This inflammatory reservoir has also suggested being a possible initiator in the transformation into a bone destructive lesion [8]. Our findings supports this only to some extent, since there was no significant difference between the expressions of bone destruction related MMPs between the HDF's and DC's. This may also have clinical relevance since rationale for symptomless wisdom tooth removal is often justified by radiologic findings and suspected future osteolytic complications; this is not justified based on HDF and DC MMP profiles. Interestingly, it seems that the initiator may be the inflammation itself rather than epithelial remnants of the follicle since it has also been demonstrated that HDF supernatants start to express high levels of MMP-9 if stimulated with TNF-α [8]. However, our new findings suggest that in the case of DC the more bone destructive nature cannot be explained by higher expression of MMPs in the proliferating epithelial interface between cyst and bone leaving the mechanisms to be discovered. It is true that our patient sample is rather small, but knowing this the statistical analysis was performed so that the strong positive samples (value 3) were also compared to low scoring samples (values 0-2) independently between the DC and HDF groups and not even then the statistical analysis did not bring any difference between the groups indicating that MMP or TIMP-1 expressions seem not to correlate the clinical nature of the entity. Now used immunostainings give reliable and functional MMP and TIMP profiles but naturally only for the selected MMPs and TIMPs. Microarray techniques could provide further knowledge about the protease profiles of DC and HDF outside MMP spectrum but this remains to be investigated.

The change of DC to malignant form is a rarity, however, several carcinoma subtypes such as epidermoid, mucoepidermoid and intraosseous carcinomas have been reported [1,2,19,20]. Of these subtypes the intraosseous carcinoma seems to be the most common one. In oral carcinomas the role of MMPs have been identified for over a two decades [21]. However, the roles of different MMPs in normal physiology are also significant which cause problems with side significant side effects and for this reason the clinical applications are still to be awaited [22]. For this reason several selective spectrum MMP inhibitors and diagnostic tools have been also designed to target distinct MMPs, especially gelatinases, in oral car cinomas [22-25]. Interestingly, in intraosseous carcinomas the role of MMPs is considerably less clear which may also relate to its embryonic origins similar to dentigerous cysts.

The protease hypothesis of cyst expansion has been tempting explanation since especially matrix metal loproteinases have been known to degrade most if not all bone matrix components. These enzymes seem to take part in the bone destruction in more aggressively behaving lesions such as keratocysctic odontogenic tumours and ameloblastomas since their MMP levels are clearly higher when compared to DC [3,14,15]. This suggests the cyst wall being the active counterpart in bone destruction and remodeling. In the case of DC and HDF MMPs clearly have a role but our new findings, however, suggest that in the case of DC other osteolytic mechanisms probably explain the expanding nature of the DC's since MMP levels did not differ. Whether this can be explained by other proteolytic mechanisms or cyst initiated indirect proteolytic mechanisms through osteoclast activation remains to be investigated.

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