Expression of cathepsin-d in odontogenic cysts and tumors

The expression of cathepsin-D in odontogenic cysts and tumors: an immunohistochemical study

Abstract

Aim: Cathepsin-D, a protease, which is an invasion promoter and plays a central role in solid tumors including oral cancer. Our aim of the study was to look for their expression pattern in epithelium and stroma of odontogenic cysts and tumors and correlate their aggressiveness to the staining intensity.

Methods: To elucidate the expression patterns of this marker, we examined immunohistochemically on formalin fixed, paraffin embedded sections of 24 odontogenic cysts and 10 odonogenic tumors, which are received for histopathologic examination in the department of oral pathology, the Oxford Dental college and hospital, Bangalore.

Results: The epithelium of granular cell ameloblastoma and odontogenic keratocyst showed maximum staining, with spillage of stained material in the connective tissue wall and at the separation of epithelium to capsule in odontogenic keratocyst, compare to other cysts and tumors.

Conclusions: Cathepsin-D could be one of the enzyme important in separation of epithelium and connective tissue in odontogenic keratocyst which helps in recurrence and intense expression in granular cell ameloblastoma with spillage into stroma, compare to other odonogenic tumors may explain its aggressive behavior, recurrence and metastatic potential. To further validate our findings it is suggested to use more sample size and monoclonal antibody for cathepsin-D.

Key words: Cathepsin-D, odontogenic cysts, odontogenic tumors, immunohistochemistry.

INTRODUCTION:

Odontogenic cysts and tumors constitute an important aspect of oral and maxillofacial pathology. Odontogenic cysts are encountered relatively common in dental practice and tumors by contrast are uncommon lesions. These lesions are of clinical significance because of their biological behavior. Various attempts to categorize morphological features to relate the biological activity have been made over the years [1] . It is well established that the cysts of histologenic labeling of odontogenic keratocyst are more aggressive tending to behave more like a sub-malignant tumor [1-6] . It has also been suggested that cysts other than odontogenic keratocyst showing keratinization if not more locally aggressive tend to have a pre-disposition to neoplastic change [7] . There have been attempts to correlate follicle size with aggression in ameloblastoma and morphologically different granular cell variant has been known to be more clinically aggressive, showing metastatic potential [8] . Numerous studies on the enzyme histochemistry of odontogenic cysts and tumors have been conducted over the years for the expression of oxidative enzymes NADH2 and NADPH2, G6PD, glutamate dehydrogenase, acid phosphates, leucineamino peptidase and ATPase [9, 10] . The epithelial lining of all the varieties of cysts showed a weak reaction for leucineamino peptidase a lysosomal protease, but there was a strong positivity in the lamina propria of odontogenic keratocyst. Similar studies on follicular ameloblastoma have showed ATPase activity in the peripheral and central cells of the follicle [9] . Based on these we made an attempt to study the

expression of cathepsin-D in odontogenic cysts and tumors, by grouping them into locally aggressive and non-aggressive based on their clinical and radiographic features.

Cathepsin-D is a proteolytic enzyme that belongs to a family known as aspartic proteases. Many homologies in the amino acid sequence have been shown to exist among the members of this group of enzymes, which includes pepsin, gastricin and rennin. Like other enzymes cathepsin-D has been shown to be synthesized in the precursor form. The enzyme itself is a glycoprotein of approximate molecular weight 52 KD and has an optimum pH of 3. 5. Cathepsin-D was present in many of the normal tissue including epithelium, fibroblast and macrophages [11] . The physiologic role of cathepsin-D is believed to be involved in self-destruction of senescent or damaged epithelial cells [12] . As cathepsin-D is an intracellular lysosomal aspartic protease apart from its role in protein catabolism through the degradation of endocytosed protein. Cathepsin-D has attracted clinical attention because of it’s over expression in variety of diseases. Increased levels of these enzymes have been reported to be an indicator of aggressive behavior in human tumors including oral squamous cell carcinoma [13] .

MATERIALS AND METHODS:

Tissue used in the study was biopsy material submitted to department of oral pathology, The Oxford Dental College, Hospital and Research centre, Bangalore. Total sample size taken was from 34 patients which comprised of 9 Ameloblastoma (1 plexiform unicystic ameloblastoma), 7 odontogenic keratocyst, 1 adenomatoid odontogenic tumor, 11 Radicular cysts and 6

Dentigerous cysts which were grouped into locally aggressive and non aggressive based on their clinical and radiologic features like size and extent of lesion, peripheral cortication, scalloping and root resorption.

*This particular radicular cyst was an extensive lesion extending from the maxillary canine to the third molar extending into and destroying the maxillary sinus and had caused root resorption from canine to second molar without causing any bony expansion. The initial clinical impression was that of a malignancy arising in the maxillary sinus.

METHODOLOGY:

Formalin fixed paraffin embedded sections of odontogenic cysts and tumors were stained by hematoxylin and eosin stain, the serial sections of the same was studied by Immuno histochemistry procedure using cathepsin-D and observed under the microscope for the intensity of cathepsin-D staining expression or non- expression. Controls were prepared by omitting primary antibody.

A grading system for intensity of expression was devised and used.

Antibody used:

1. Polyclonal rabbit anti-human primary cathepsin-D, 7ml ready to use (DAKO Corporation N1625). Denmark
2. Biotinylated anti-mouse, anti-rabbit, anti-goat Igs, LINK/secondary antibody, 15 ml ready to use. (DAKO LSAB+ system, K0679).
3. Streptavidin conjugated to horseradish peroxidase. (DAKO LSAB+ system, K0679).
4. Liquid Diamino benzidine chromogen.

OBSERVATION AND RESULTS:

All odontogenic cysts and tumors were observed for intensity of cathepsin-D stain in epithelium and stroma/ connective tissue capsule by categorized into mild, moderate and marked staining. Statistical analysis was done using students T test. Table 1 shows number of cases in which cathepsin-D shows mild, moderate and marked staining in various epithelial layers and stroma. Table 2 shows statistical relation of staining intensity of cathepsin-D in each layer and stroma/capsular wall between each odontogenic cysts. Table 3 shows statistical relation of staining intensity of cathepsin-D in each layer and connective tissue stroma between each odontogenic tumors.

DISCUSSION

The idea of immunohistochemistry staining for a lysosomal protease cathepsin-D in odontogenic cysts and tumors of varying biological behavior pattern was with the hope that it could contribute to a better understanding of metabolic processes that are responsible for that behavior. Traditionally we have always focused on the epithelium in odontogenic cysts and epithelial tumors. Much like the mesmerizing effect of giant cells in giant cell lesions, the epithelium in odontogenic cysts and epithelial tumors has held a magnetic quality for research workers. The epithelial component dictates the diagnosis, but the role of connective tissue wall and the stromal cells in tumors has not always been given due consideration. The epithelium is not always at the advancing front of these lesions as is especially seen in case of cysts. In this study in addition to the epithelium we also looked at the expressivity of cathepsin-D in the connective tissue and stromal cells.

In granular cell ameloblastoma we observed marked staining pattern in the cytoplasm of the granular cells, often spilling into the connective tissue which may contribute to the aggressive nature of the lesion and its propensity for metastasis (Fig 1a & 1b). As compared to the granular cell ameloblastoma other odontogenic tumor types such as follicular, unicystic, plexiform ameloblastoma and adenomatoid odontogenic tumor (Fig 2a &2b) showed less intense staining pattern and the staining was restricted to cytoplasm of these epithelial cells with minimal stromal staining. Apart from the granular cell ameloblastoma we could not derive any correlation between clinical behaviour and cathepsin-D expression. Among the 3 cyst types we found a characteristic epithelial staining pattern in odontogenic keratocyst in comparison to radicular and dentigerous cysts. Among 7 odontogenic keratocyst only one case showed superficial granular staining of the epithelial cells with no separation of epithelium from connective tissue. In all other cases we observed granular staining through the full thickness of the epithelium, more in the basal and supra-basal layers, with intense/marked staining at the region of separation of epithelium from connective tissue with granular staining pattern in separation zone (fig 3a &3b).

In dentigerous cysts there was only superficial staining of epithelium. The radicular cysts showed uniform staining in the entire length of epithelium (fig4). In the one radicular cyst which was clinically more aggressive; a similar pattern of staining was observed. Though the epithelial staining in radicular cysts was almost similar to that seen in odontogenic keratocysts we did not find any areas of cleavage between epithelium and connective tissue.

In the odontogenic keratocyst the staining pattern though similar to the radicular cysts, in the area of split the staining was very intense, and some stained material was noticed in the space between the epithelium and the connective tissue leading to the speculation that the increased expression may contribute to the split, which may have prognostic consequences in terms of recurrence by way of cleaving of epithelium at the time of attempted enucleation or biopsies .

In addition to variations in staining patterns of the epithelial lining of the different types of cysts, their walls showed variation in staining from the epithelial end to the bony end . All the cyst types showed expressivity in the immediate sub-epithelial region as well as the bony end of the cyst wall. The intensity of staining progressively increased from the dentigerous cyst through the radicular cyst to the odontogenic keratocyst. The intermediate zone showed relatively scanty expression. This pattern of increasing expression seemed to correlate with increasing aggression. The one radicular cyst grouped in the list of aggressive lesion showed intense staining in the most peripheral areas similar to that seen in the odontogenic keratocyst. All the inflammatory cells seen in connective tissue wall and keratin of the surface layer and granules of granular layer of odontogenic cysts showed intense staining.

To the best of our knowledge this is the first study on expression of cathepsin-D in odontogenic cysts and tumors although studies on various other lysosomal enzymes like leucineaminopeptidase etc have been published. Hence it may be presumptuous on our part to make claims on the role of cathepsin-D in aggressive behaviour of odontogenic cysts and tumors,

however that there is perceptible variation in expression would suggest that additional efforts in the area may help to understand the metabolic processes that lead to aggressive behaviour. Another area open for exploration is precystic epithelium as in the case of periapical granulomas and the role of these enzymes in cystogenesis.

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