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"An Immunohistochemical Study" of Parathyroid Hormone - Related Protein: A Comparative Analysis In Ameloblastoma and Dentigerous Cyst

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Abstract: It has been pointed out that Parathyroid Hormone related Protein (PTHrP) secreted by the developing enamel epithelium targets receptors in overlying bone, thereby activating bone resorption and allowing tooth eruption. Accordingly, it is conceivable that ameloblastoma, which to a degree recapitulates certain characteristics of enamel epithelium, would also express PTHrP. This study is done to assess the PTHrP expression in ameloblastoma, to investigate its role in local bone resorption and provide explanation for the infiltrative growth and destructive behavior of this tumor which may hold significant therapeutic implications. A group of 50 histo-pathologically diagnosed, formalin-fixed, paraffin embedded tissue samples, consisting of 30 samples of ameloblastoma and 20 samples of dentigerous cyst were collected from the Department of Oral and Maxillofacial Pathology, Govt. Dental College, Bangalore, and were subjected to immunohistochemical methods using anti PTHrP Antibody with indirect immuno-enzyme Labeled StreptAvidin Biotin method (LSAB). Out of 30 cases of ameloblastoma 26(86.7%) cases showed positivity for PTHrP. Among the 20 cases of dentigerous cysts 9(45%) cases showed positivity for PTHrP expression. When we compared the percentage of cases showing positivity for PTHrP expression in ameloblastoma (86.7%) and dentigerous cyst (45.0%) we obtained a statistically significant p-value of 0.0012. The current study may suggest increased PTHrP expression by ameloblastoma may play a significant role in local bone resorption and may provide explanation for the infiltrative growth and the destructive behavior of this tumor.

Key words: Ameloblastoma, dentigerous cyst, immunohistochemistry, PTHrP

INTRODUCTION

Ameloblastoma is a slow growing and locally invasive odontogenic tumor with a potentially destructive behavior and high rate of recurrence. Philbric *et al*. (1998) pointed out that expression of PTHrP in enamel epithelium to be necessary for tooth eruption. Accordingly, it is conceivable that ameloblastoma which to a degree recapitulates certain characteristics of enamel epithelium would also express PTHrP. Therefore it appears that PTHrP expression by ameloblastoma targets receptors in the surrounding bone subsequently causing resorption and facilitating tumor growth (Philbrick *et al*., 1998). This may account for the locally aggressive behavior of ameloblastoma. This study is done to assess the role of PTHrP expression on the aggressive behavior of Ameloblastoma, by comparing the same in clinically less aggressive dentigerous cyst.

MATERIALS AND METHODS

Fifty formalin fixed paraffin embedded tissue blocks were collected from the files of Department of Oral and Maxillofacial Pathology, Govt. Dental College and Research Institute, Bangalore, where this study was conducted during the period of 2007-2008. Serial sections of 4 µm thickness were taken. The endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 15 min. The slides were then immuno stained using anti PTHrP monoclonal mouse antibody (Calbiochem- Merck laboratories, Germany-Epitope is within 38-64 amino acid sequence of PTHrP). For negative control, Tris Buffer Solution (TBS) was used instead of the primary antibody. Slides were then treated with Link Secondary antibody. Peroxidase conjugated streptavidin biotin method was used and brown staining was considered positive. The slides were stained lightly with Harris haematoxylin, washed gently under running water for 10 min. They were dehydrated and dipped in xylene and mounted in DPX, a non-aqueous permanent mounting medium using coverslips.

Interpretation of staining: Presence of brown colored end product at the site of target antigen was indicative of positive reactivity. Depending on the intensity and distribution of brown stain, grades were assigned as Mild,Moderate and high. A mild (+) immunostaining reaction score was assigned to cases demonstrating faint

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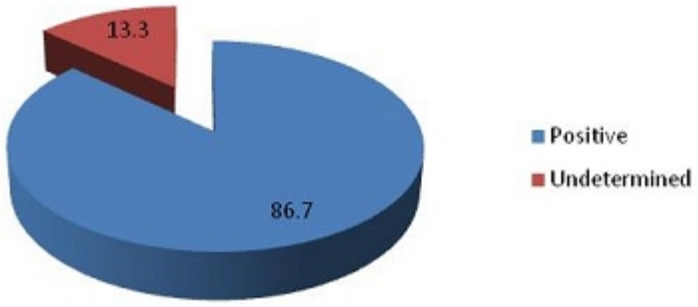

Fig. 1: Distribution of PTHrP in Ameloblastoma

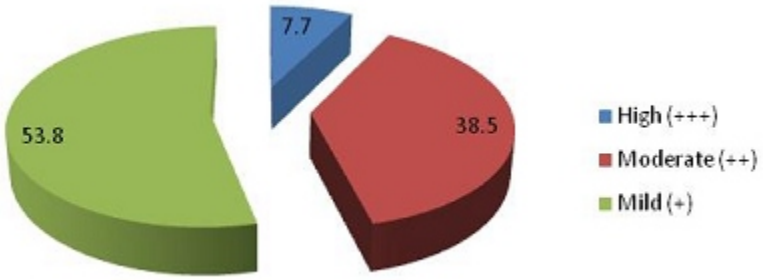

Fig. 2: Intensity of PTHrP expression in Ameloblastoma

brown cytoplasmic stain distributed in patchy, non contiguous zones of the neoplastic epithelium.

A moderate (++) immunostaining rate was assigned to cases displaying darker brown cytoplasmic staining in diffuse areas but not involving the entire neoplastic epithelial tissue.

A high (+++) immunostaining reaction score was assigned to cases showing dark, dense cytoplasmic staining involving most or all of the neoplastic epithelium in tissue sections.

The negative control tissue demonstrated absence of specific staining. Squamous cell carcinoma was used as the positive control. For Negative control sections, the primary antibody was omitted during immunohistochemical staining.

The data collected was entered in Microsoft excel and statistical analyses were performed using the Statistical Package for Social Science (SPSS (ver. 10.5)) software. The results were presented in the number and percentage in Tables and Figures. Proportion was compared using Chi-square test. A p-value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

A total of 50 cases including 30 cases of ameloblastoma and 20 cases of dentigerous cyst were selected and studied for the expression of PTHrP. The distribution of PTHrP expression in ameloblastoma is summarized in Fig. 1.

Out of 30 cases of ameloblastoma 26(86.7%) cases showed positivity for PTHrP. We were unable to determine the positivity or negativity for 4 (13.3%) cases as the staining was not convincing. Out of 26 cases of positive PTHrP expression cases 2(7.7%) cases showed high (+++) intensity of expression, 10 (38.5%) cases showed moderate (++) intensity of expression and 14 (53.8%) cases were showing mild (+) intensity of expression (Fig. 2).

Among the 20 cases of dentigerous cysts 9 (45%) cases showed mild positivity for PTHrP expression and 11(55%) cases were negative as in Fig. 3.

When we compared the percentage of cases showing positivity for PTHrP expression in ameloblastoma (86.7%) and dentigerous cyst (45.0%), we obtained a p-value of 0.0012, which is statistically significant (Fig. 4).

Immunohistochemical reactivity for PTHrP: Immunohistochemical reactivity for PTHrP was detected in the cytoplasm of both neoplastic epithelial odontogenic cells and epithelial cells of dentigerous cyst (Fig. 5 and 6).

In ameloblastoma, follicular variant peripheral columnar cells and central stellate cells showed similar reactivity. In acanthomatous variant peripheral cells and stellate cells showed reactivity to PTHrP but not the

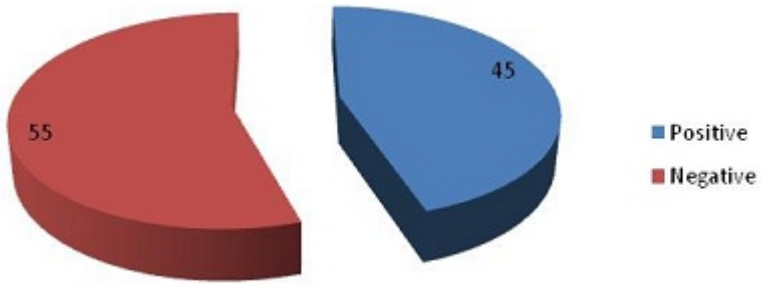

Fig.3 : Distribution of PTHrP expression in Denitgerous Cyst

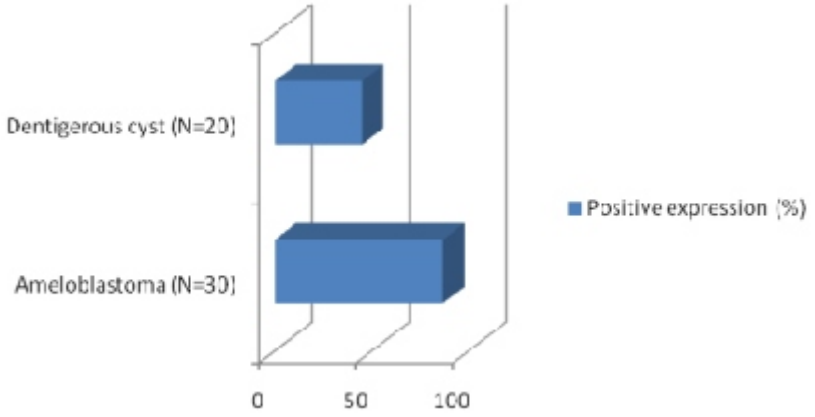

Fig. 4: Comparision of Positive expression of PTHrP in Dentigerous cyst and Ameloblastoma

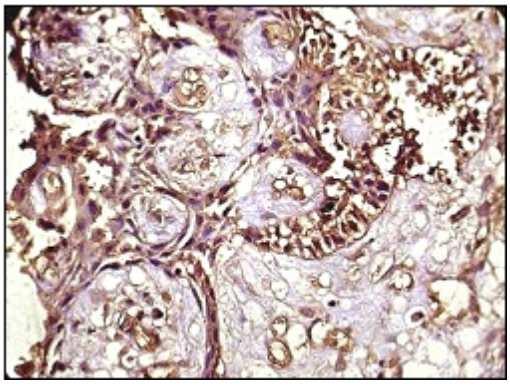

Fig. 5: Ameloblastic Epithelial cells showing high degree of positivity for PTHrP.

central squamous metaplastic cells (Fig. 7). Desmoplastic ameloblastoma showed similar results.

PTHrP reactivity was detected in fibrous tissue capsules of four out of 26 ameloblastoma cases. In Unicystic ameloblastoma basal columnar cells showed more reactivity than the superficial stellate cells. Areas showing squamous metaplasia were not showing reactivity to PTHrP. Cystic epithelial lining of dentigerous cyst showed reactivity to PTHrP of mild intensity. In all the cases that we studied dentigerous cysts did not show any reactivity of fibrous capsule.

DISCUSSION

PTHrP is found in normal keratinocytes, lactating mammary tissue, placenta, parathyroid gland, the central nervous system, and in a number of other sites suggesting a widespread physiologic role (Shear, 2002; Merendino *et al*., 1986; Broadus and Stewart, 1994; Danks *et al*., 1989). It locally regulates tissue functions in contrast to the systemic hormonal effect of PTH (Shear, 2002). PTHrP is also expressed by different epithelia, including epidermis and mammary gland, and it participates in epithelial-mesenchymal tissue interactions by activating receptors for PTH and PTHrP in mesenchyme (Shear, 2002; Pioszak *et al*., 2009). PTHrP expression has been reported in-patients with breast carcinoma with metastatic bone deposits, and in-patients with multiple myeloma with hypercalcemia (Powel *et al*., 1991; Bouizar *et al*., 1992; Strewler and Nissenson, 1990; Burtis *et al*., 1990). This ligand binds

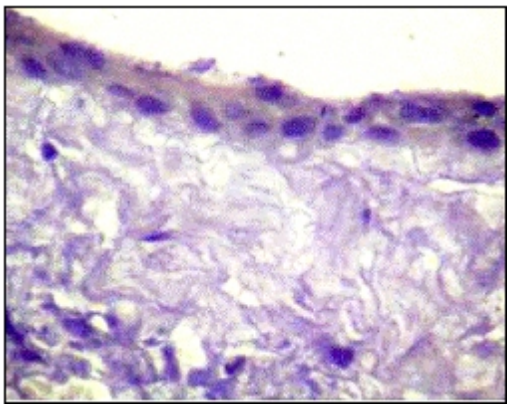

Fig. 6: Epithelial lining of dentigerous cyst showing very mild degree of positivity

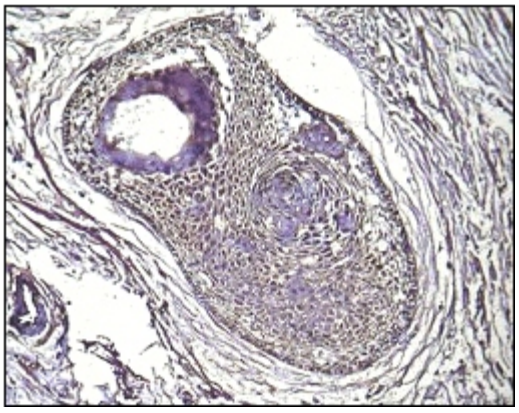

Fig. 7: Ameloblastic Island showing moderate degree of positivity. Acanthotic area negative for PTHrP

to PTHrP receptors on osteoclasts subsequently stimulating bone resorption. When bone is resorbed, transforming growth factor β (TGF β) and other peptides, such as insulin-like growth factor get released and stimulate production of PTHrP by proliferating cancer cells, thereby establishing a vicious cycle (Abdelsayed *et al.*, 2004). PTHrP might contribute to cancer progression by stimulating cancer cell growth and/or inhibiting apoptosis. PTHrP-(1-34) is a growth stimulant in many malignant cell-lines including human lung cancer cells, breast carcinoma cells, renal cell carcinomas and it inhibits apoptosis in various cells, including growth plate chondrocytes (Shear, 2002; Amizuka *et al.,* 1996; Amling *et al.*, 1997; Wu *et al.*, 1996; Strewler, 2000).

Observation in patients with bone metastases suggest that breast cancer cells in bone expresses PTHrP more frequently than in soft tissue sites of metastasis or in the primary tumor. The capacity of breast cancer cells to express PTHrP may give them a growth advantage after they have metastasized to bone, due to the ability of PTHrP to increase osteoclastic bone resorption (Guise *et al.*, 1996; Bouizar *et al.*, 1992; Burtis *et al.*, 1990; Liao and McCauley, 2006).

The evidence implicating PTHrP expression in odontogenic epithelium comes from several lines of studies by Philbrick *et al.* (1998), Shear (2002), Abdelsayed *et al.* (2004), Kumamoto and Ooya (2004), Li *et al.* (1997) and McGuirt *et al.* (1981). Shear (2002) investigated the immunocytochemical expression of PTHrP in odontogenic cysts. Using paraffin sections and two antibodies to PTHrP they found that all the OKCs (n = 10), nine out of 10 dentigerous and eight out/10 radicular cysts showed reactivity for PTHrP localized mainly to the basal and suprabasal cells. However, the OKC linings expressed significantly higher levels than those of the dentigerous ($p<0.003$) and the radicular cysts.

Li *et al.* (1997) have shown that PTHrP is found in a variety of odontogenic cysts, especially in Odontogenic keratocysts, and suggest that PTHrP modulates cyst expansion and bone resorption (Guise *et al.*, 1996). Expression of PTHrP has been confirmed during the tooth development, and PTHrP gene knock out mice have shown disturbed tooth development, suggesting that this is involved in the morphogenesis and eruption of teeth (Philbrick *et al.*, 1998; Abdelsayed *et al.*, 2004; Kumamoto and Ooya, 2004).

Philbrick *et al.* (1998) pointed out that expressions of PTHrP in enamel epithelium to be necessary for tooth eruption. Accordingly, it is conceivable that ameloblastoma, which to a degree recapitulates certain characteristics of enamel epithelium would also express PTHrP. Therefore it appears that PTHrP expression by ameloblastoma targets receptors in the surrounding bone subsequently causing resorption and facilitating tumor growth. This may account for the locally aggressive behavior of ameloblastoma - a characteristic that seems similar to the PTHrP mediated bone destruction reported in breast carcinoma metastatic to bone. PTHrP expression by all cases, regardless of microscopic subtype, suggests that it is involved in bone resorption, presumably facilitating growth of ameloblastoma. In a study by Abdelsayed *et al.* (2004), intense PTHrP immunoreactivity was seen exclusively in 9 cases of conventional ameloblastoma, whereas none of the unicystic ameloblastomas or ameloblastomas arising in dentigerous cyst showed such levels of reaction.

In our series of cases of ameloblastoma, all cases exhibited PTHrP expression with various levels of immunoreactivity.

Immunohistochemical reactivity for PTHrP was detected in the cytoplasm of neoplastic epithelial odontogenic cells. In follicular and plexiform variants of ameloblastoma peripheral columnar cells and central stellate cells showed moderate to intense reactivity.

Unicystic ameloblastoma showed mild to moderate intensity of PTHrP expression. This observation may aid in explaining the finding of greater bone destruction associated with conventional ameloblastoma, relative to the less aggressive behavior observed in unicystic tumors. In acanthomatous variant peripheral cells and stellate cells showed reactivity to PTHrP but not the central squamous metaplastic cells. In a study by Kumamoto and Ooya (2004), he suggested that PTHrP might be associated with tumor cell differentiation in ameloblastoma as he found slightly stronger expression of PTHrP, especially in keratinizing cells, in acanthomatous ameloblastomas (Kumamoto and Ooya, 2004). This differs from our observation where acanthotic areas of epithelial islands are not expressing PTHrP, which may suggest the down regulation of the protein after the terminal differentiation of the cell.

Desmoplastic ameloblastoma showed moderate expression coinciding with the results of previous researchers (Abdelsayed *et al*., 2004).

In a study by Li *et al.* (1997) PTHrP reactivity was detected in fibrous tissue capsules of all the odontogenic cysts with those of odontogenic keratocyst subjectively showing the most intense reactivity. Endothelial cells, fibroblasts and inflammatory cells within the cyst capsules showed consistent staining that was specifically removed in peptide neutralization experiments. In some sections, collagen, nerve fibres, and smooth and skeletal muscle also appeared to show weak but specific reactivity for PTHrP (Li *et al*., 1997). In a study by Philbrick *et al.* (1998) and Strewler (2004), positive signaling was seen in the connective tissue stroma of a case of ameloblastoma (Abdelsayed *et al*., 2004).

In our study, positive signaling was seen in the connective tissue stroma of 4 ameloblastoma cases. Although definite conclusions could not be drawn, we propose that PTHrP production by the supporting stroma may be induced through tumor epithelium - connective tissue interaction. Low-level constitutive stromal production of PTHrP, and low-level PTHrP secretion by tumor epithelium into stroma, remain possible explanations for this finding.

In the present study, among the cases of dentigerous cysts, 9/20 cases showed mild positivity for PTHrP expression correlating with previous studies done by Li *et al.* (1997) and Kumamoto and Ooya (2004).

CONCLUSION

In this study when a comparison was made between the percentages of cases showing positivity for PTHrP expression in ameloblastoma and dentigerous cyst a statistically significant result ($p = 0.0012$) was obtained. The results obtained clearly correlate with the clinical behavior of the two odontogenic lesions one (ameloblastoma) being aggressive showing high recurrence and the other (dentigerous cyst) with a milder clinical course.

Another significant finding in this study is the down regulation of PTHrP expression in terminally differentiated keratinizing cells in acanthomatous ameloblastoma. Clinical and biological significance of this finding is yet to be elucidated through further studies in this field.

The different amounts of PTHrP detected in both the linings of ameloblastoma and dentigerous cysts and fibrous walls of few cases of ameloblastoma indicate that local production of PTHrP by epithelial and mesenchymal cells contributes to their differing clinical behavior, pattern of growth and bone resorbing capacity and has a significant role in treatment plan.

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