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Original Article

Dentistry Section

Comparision of Immunohistochemical Expression of CD10 in Odontogenic Cysts

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ABSTRACT

Background: Expression of CD10 has been documented in various tumors like nasopharyngeal carcinoma, gastric carcinoma, squamous cell carcinoma, odontogenic tumors.

Aim: To evaluate and compare CD10 expression in odontogenic cysts like radicular cyst, dentigerous cyst and odontogenic keratocyst (OKC).

Materials and Methods: Total 60 cases were included in the study, comprising 20 cases each of radicular, dentigerous and odontogenic keratocyst. Each case was evaluated and compared for immunohistochemical expression of CD10. Results obtained were statistically analysed using ANOVA test followed by post hoc test Tukey-Kramer Multiple Comparisons Test for continuous variable and Chi-square test for discrete variable.

Results: More number of cases showing sub-epithelial stromal CD10 expression were found in OKC among the cysts.

Conclusion: CD10 expression was more in OKC compared to radicular and dentigerous cysts.

Keywords: CD10, Dentigerous cyst, OKC, Radicular cyst

INTRODUCTION

Odontogenic cysts represent the commonest form of cystic lesions that affect the human skeleton and are derived from the epithelium associated with the development of the dental apparatus. After completion of tooth formation in the jaws, odontogenic epithelium breaks down and persists as cell-rests, which under the influence of suitable stimuli can proliferate and give rise to odontogenic cysts. Epithelial lining of these cysts may be derived from proliferating root sheath residues, reduced enamel epithelium, remnants of dental lamina or possibly from basal layer of oral epithelium [1-2].

These cysts are histologically simple structures, lined by stratified squamous epithelium and have a fibrous tissue wall exhibiting a variable degree of inflammatory cell infiltration [1-2].

Different odontogenic cysts vary in their pattern of occurrence, recurrence and prognosis. Some of odontogenic cysts like OKC is known for its recurrence even after surgical excision and also has a neoplastic nature [3]. But the same type of behaviour is not seen with other odontogenic cysts like radicular cyst. This type of peculiar behaviour of these lesions need to be further studied with appropriate markers which may help us in understanding their biological behaviour and underlying mechanisms behind them. Several immunohistochemical markers have been used to study these cysts and CD10 is one of the stromal cell markers used to study the aggressiveness of these lesions.

CD10 is 90-110 kDa cell surface zinc dependent metalloprotease which possesses a well-defined enzymatic activity where it cleaves and inactivates neuropeptides and peptide hormones at the amino terminus to hydrophobic residues within the peptide sequences, thereby decreasing the cellular response to local peptide hormones. It has also been called as neutral endopeptidase, enkephalinase, neprilysin and common acute lymphoblastic leukemia antigen (CALLA) [4].

CD10 is expressed on some normal and neoplastic haemopoietic, lymphoid and epithelial cells [5]. CD10 expression has also been detected in tumor associated stromal cells indicating its vital role in tumor-stromal interactions [6]. Many studies on the role of CD10 has been done on OKC, dentigerous cyst and ameloblastoma, but not on radicular cyst. Hence the present study is carried out to know the expression of CD10 in odontogenic cysts like radicular cyst, dentigerous cyst and OKC and evaluate its potential role in aggressiveness of these cysts.

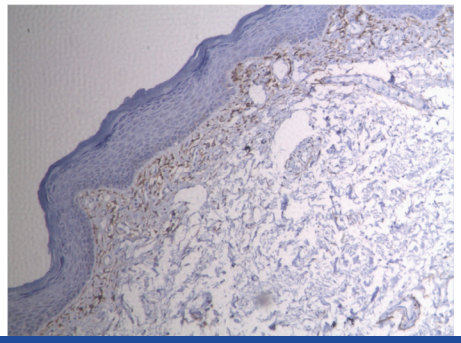
MATERIALS AND METHODS

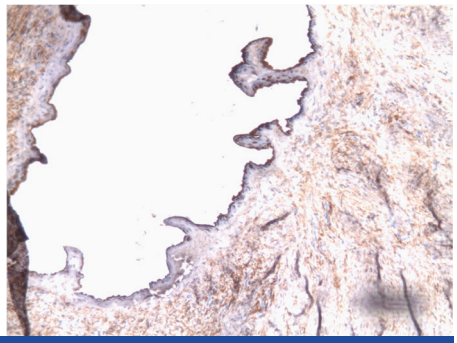
The material for the study included 60 formalin-fixed, paraffin-embedded tissue blocks retrieved from the Department of Oral and Maxillofacial Pathology, Sri Sai College of Dental Surgery, Vikarabad. These comprised of 20 cases each of radicular cyst, dentigerous cyst and odontogenic keratocyst (OKC). Ten normal lymph nodes have been used as a positive control group for CD10 staining. All these cases were diagnosed by routine haematoxylin and eosin staining and then subjected to immunohistochemical staining for CD10.

The antibodies and reagents used for immunohistochemical technique were obtained from Scytek laboratories (Logan, Utah, USA) ready to use kit [Table/Fig-1] which consists of Primary antibody-CD10, specific mouse monoclonal antibody (Clone 56C6, Isotype IgG1), Secondary antibody - Goat antipolyvalent IgG, Peroxide block, Conjugate - Horse Radish Peroxidase, Chromogen substrate - Diaminobenzidine tetra hydrochloride (DAB).

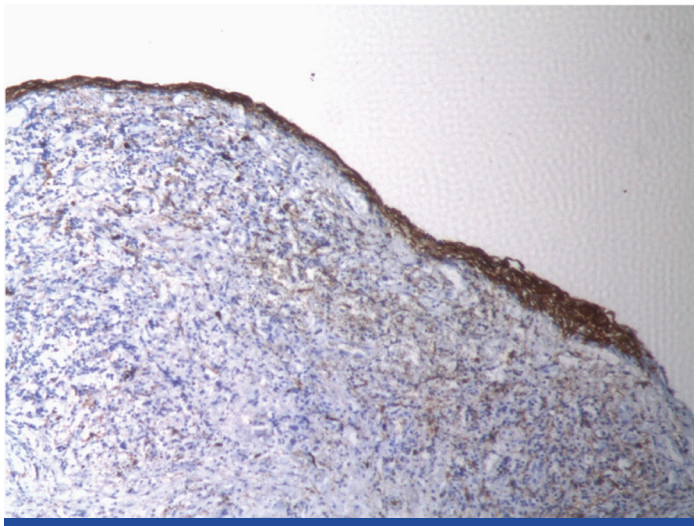
Buffers used in the method consisted of 0.1M citrate buffer for antigen retrieval and phosphate buffered saline (PBS) for washes in between the steps.

4µ thick sections were taken onto poly-L-lysine adhesive coated micro slides and incubated for one hour at 65 degree centigrade on a slide warmer for proper adhesion of the section to the slide. Sections were dewaxed through three changes of xylene, five minutes each and hydrated through descending grades of alcohol (100%, 90%, 70%) five minutes each and brought to distilled water. Antigen retrieval is done by using microwave oven method where sections were incubated in 0.1 M citrate buffer for 15 minutes comprising of three cycles of five minutes each. Then sections were allowed to cool down to room temperature and then washed in distilled water. For endogenous peroxidase blocking, micro sections were then incubated with hydrogen peroxide for 15 minutes. After this, sections were washed in phosphate buffered saline (PBS), two changes of five minutes each. For blocking of non-specific





[Table/Fig-1]: Ready to use Kit from scytek laboratories, logan, utah, usa **[Table/Fig-2]:** Basal cells of epithelium and sub-epithelial stromal cd10 expression in odontogenic keratocyst **[Table/Fig-3]:** Superficial cells of epithelium and stromal cd10 expression in dentigerous cyst



[Table/Fig-4]: Intense epithelial and stromal cd10 expression in radicular cyst

were > 10%, the specimen was considered to be positive and when the stromal cells positivity was < 10%, the specimen was classified as CD10 negative [7]. The intensity of staining was graded as weak and strong by comparing with lymphocytes.

STATISTICAL ANALYSIS

The CD10 stromal cells were evaluated in cysts in 10 high power fields in each case and expressed as percentage. The count and staining intensity was assessed by two investigators simultaneously using a double headed light microscope. Both had to agree on the count and intensity of staining for positive cells.

Comparisons between stromal cell immunoreactivity, intensity of staining for CD10 and the mean percentage of positive cells were evaluated. Descriptive statistics such as mean, SD and percentage was used. Comparison between groups was done using ANOVA test followed by post hoc test Tukey-Kramer Multiple Comparisons Test for continuous variable and Chi-square test for discrete variable. A p-value less than 0.05 were considered as significant.

CD10 Immunoreactivity in Stromal Cells	Radicular Cyst	OKC	Dentigerous Cyst	Test statistic value	p-value	Remarks
Mean percentage of positive cells (mean +SD)	5.63(2.07)	14.75(9.6)	5.5(2.49)	16.29	p<0.0001	Significant
Intensity of staining Weak Strong	 12 (60%) 8 (40%)	 8 (40%) 12 (60%)	 14 (70%) 6 (30%)	3.8	p=0.15	Non significance
No of cases with CD-10 expression > 10% cells	2/20 (10%)	14/20 (70%)	2/20 (10%)	22.86	p<0.0001	Significant

[Table/Fig-5]: Immunohistochemical expression of cd10 in sub-epithelial stromal cells of odontogenic cysts
**post hoc test tukey-kramer multiple comparisons test applied, radicular cyst vs okc, p<0.001; radicular cyst vs dentigerous cyst, p>0.05; okc vs dentigerous cyst, p<0.001*

background staining sections were treated with 10% bovine serum albumin at room temperature for 10 minutes.

Sections were then incubated with primary antibody (CD10) for 60 minutes in a humid chamber. For reagent control, no primary antibody was added. Sections were then washed in three changes of PBS thoroughly for five minutes each. Sections were then incubated with secondary antibody (goat antipolyvalent IgG) at room temperature for 30 minutes followed by wash in four changes of PBS for five minutes each. Then sections were incubated with horse radish peroxidase conjugate for 30 minutes and washed in four changes of PBS for five minutes each.

For visualization, sections were then incubated with diaminobenzidine tetra hydrochloride (DAB) for 10 minutes. Sections were then lightly counterstained using Meyer's haematoxylin for 60 seconds and gently washed in running tap water for two minutes.

Evaluation of the staining for CD10: Assessment of CD10 positive cells was performed using double headed light microscope at 40X. The criteria used to define CD10 positive cells were

- Brown staining of cytoplasm or cell membrane of stromal cells
- Brown staining in lymphocytes

The CD10 immunostained stromal cells subepithelially in the cyst group were evaluated in 10 high power fields (HPF) in each case and expressed as percentage. When stromal cells positive for CD10

RESULTS AND OBSERVATIONS

In the present study, the control sections showed CD10 expression in germinal centres of lymphnodes. CD10 expression was also evaluated in different odontogenic cysts. Immunohistochemical expression of CD10 was observed in cytoplasm and cell membrane of stromal cells. In each case, 10 high power fields (HPF) were selected and evaluated for CD10 expression. The intensity of staining was also graded as weak or strong.

The mean percentage of CD10 positive cells was highest in OKC (14.65+9.6) [Table/Fig-2,3] compared to radicular and dentigerous cyst. OKC's showed higher number of CD10 positive cells compared to other cysts. Intensity of staining was strong in OKC but it was not that significant.

In DC's, superficial cells of epithelial lining were positive in three cases [Table/Fig-4] and in OKC's basal layer of the epihelium was positive in four cases and in radicular cysts entire epithelium was positive in most of the cases [Table/Fig-5].

DISCUSSION

Dentigerous cyst is the most common developmental cyst in the oral cavity and is always associated with the crown of a tooth attached to cementoenamel junction [8]. Radicular cyst is the most common inflammatory odontogenic cyst. Among different odontogenic cysts, OKC shows higher rate of recurrence and tendency for invasion in

to adjacent tissues which is similar to ameloblastoma. So the term Keratocystic Odontogenic Tumor (KOT) has been given by WHO suggesting its aggressive nature [9]. This peculiar and aggressive behaviour of OKC has been attributed to its epithelial lining [10-12]. But the importance of connective tissue wall on the pathogenesis of OKC has been proposed by Browne in [13].

CD10 is a cell surface Zinc dependent metalloprotease found on some normal and neoplastic, hemopoietic, lymphoid and epithelial cells. This wide distribution suggests a crucial role invivo of this antigen. CD10 expression on cancer cells is associated with apoptosis and proliferation and stromal expression is associated with tumor progression [8,14]. The invasive and metastatic potential of several types of neoplastic cells is regulated by interactions with stromal cells, which involve stimulatory and inhibitory factors that regulate such functions as cellular adhesion, migration and gene expression [15].

In many carcinomas like gastric carcinoma, hepatocellular carcinoma, malignant melanoma CD10 expression has been associated with increased dysplasia, tumor invasion and metastasis [16-18].

CD10 expression has also been seen in invasive ductal carcinoma, colorectal carcinoma and nasopharyngeal carcinoma [19-21].

Piatelli et al. found highly significant correlation between stromal CD10 positivity with the lymph node status, the presence of local recurrences and the histological grading and showed that CD10 expression can, perhaps have an important role in tumor invasion, probably facilitating the occurrence of metastases in oral squamous cell carcinoma [22]. Iezzi et al., showed that solid ameloblastoma cases showed significantly higher percentage of CD10 positive cells than the unicystic and peripheral ameloblastoma variants [4].

In the present study, stromal CD10 positivity was seen in higher number of cases in OKC compared to radicular and dentigerous cysts and the results are same with the studies of Azadeh Andisheh Tadbir who found more CD10 expression in OKC compared to DC [23].

Mean percentage of positive cells was also higher in OKC. Intensity of staining was strong in OKC compared to radicular and dentigerous cysts. Stromal expression of CD10 was almost similar in both radicular cyst and dentigerous cyst. Higher stromal CD10 expression in OKC might be related to the aggressive nature of OKC and reinforce the classification of OKC as Keratocystic Odontogenic Tumor(KOT). But how stromal CD10 positivity increases aggressiveness remains unresolved. Stromal expression of CD10 is associated with the malignant transformation of keratinocytes [24].

Involvement of CD10 in the acceleration of cell cycle and motility might be a probable theory. Since CD10 is structurally similar to other matrix metallo proteinases (MMPs) and stromeolysin, it may create a microenvironment that facilitate the invasion and metastsis of tumor cells [25].

Although our main aim was to study stromal CD10 expression we also found CD10 positivity in some epithelial cells of OKC and dentigerous cyst which is similar to the findings of Giovanna Iezzi who found CD10 positivity in some odontogenic epithelial cells. But CD10 was positive in basal layers of epithelium in OKC and superficial layers of dentigerous cyst. These results are in accordance with the results of Masloub et al. [26] entire odontogenic epithelium was positive in most of the cases in radicular cyst. CD10 positivity in most of the epithelium in radicular cyst might indicate some relation between inflammation and CD10.

This alteration in the expression of CD10 by the cells of the epithelial lining may be related to the proliferative potentiality of the epithelial linings of inflammatory cysts or cysts with secondary inflammation. This could possibly be due to influence of both CD10 stromal cells and inflammatory cells on the overlying epithelium. However, high CD10 expression in dentigerous cyst and OKC might indicate the potential neoplastic activity of the epithelial lining of these cysts.

Bains and Jagmohan also found CD10 positivity in epithelial cells in breast carcinoma [27]. The possible role of CD10 in odontogenic epithelial cells should not be overlooked and needs to be further evaluated with more number of cases.

Further studies on the molecular basis of CD10 expression in stromal-tumor interaction is necessary to be carried out to elucidate the role of this glycoprotein.

CONCLUSION

In the present study, more number of cases showing sub-epithelial stromal CD10 expression was found in OKC. Intensity of staining was strong in OKC compared to other cysts. So we can conclude that CD10 molecule can be used as an immunohistochemical marker to assess the aggressive nature of different odontogenic cysts. From our study, we can conclude that OKC is more aggressive than any of the other odontogenic cysts. CD10 molecule can also be used in odontogenic cysts in addition to odontogenic tumors as a immunohistochemical marker.

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