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Original Research

Comparison of calretinin expression in Odontogenic cyst and Ameloblastoma- An immunohistochemical study

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ABSTRACT:
Aim: To study the expression of calretinin in odontogenic cyst as well as odontogenic tumor and correlate the importance of the calretinin in differentiating unicystic ameloblastoma from odontogenic keratocyst. **Materials and Methods:** 20 cases each of ameloblastoma, 20 cases of unicystic ameloblastoma, 20 cases of dentigerous cyst and 20 cases of odontogenic keratocyst was assessed using calretinin. Slides were made from the archival blocks of each case and were stained immunohistochemically with calretinin. Case history performa was filled from the previously recorded case. **Results**: Correlation between calretinin staining and histopathological diagnosis was done and it was found that all 20 cases of ameloblastoma showed positivity for calretinin whereas 17% out of 20 cases of unicystic ameloblastoma showed positivity for calretinin staining. All the cases of dentigerous cyst and odontogenic keratocyst were negative for calretinin. Thus there was a significant difference in the distribution of staining for calretinin in the different odontogenic cysts/tumors(chi square value= 69.742, p value<0.01. **Conclusion**: The expression of calretinin in ameloblastoma suggest specific immunohistochemical marker for ameloblastic epithelium and serve as an important diagnostic aid in differentiating cystic odontogenic lesions and ameloblastic tumors. It was found that unicystic ameloblastoma showed a focal distribution pattern for calretinin whereas in ameloblastoma both focal and diffuse patterns were observed.
Keywords- Calretinin, Odontogenic tumor, Unicystic Ameloblastoma.

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INTRODUCTION

Odontogenic cysts and tumors are a diverse group of lesions which arise from the tooth forming apparatus or its remnant. They have variable clinical and biological behavior which depend on degree of inductive tissue interaction. Ameloblastoma is a benign, locally aggressive epithelial odontogenic tumor that has the potential to become malignant and produce metastasis to distant sites such as lungs and kidneys. OKC is an aggressive cyst with neoplastic behavior.[1,2] It is characterized, histologically by a palisaded basal cell layer of columnar cells and a surface of corrugated parakeratin, sometimes with spongiosis, resembling closely the stellate reticulum. It is usually about 5–8 cell layers thick without rete ridges formation.

In small incisional biopsies and if the cystic epithelium displays reactive changes induced by inflammation, it can closely resemble unicystic ameloblastoma histologically.[3] Thus, at times, both lesions become histologically indistinguishable.

Many techniques like demonstration of cell surface carbohydrates with blood group specificity, characterization of cytokeratin profiles, AgNOR count and quantification of cell proliferation markers like PCNA and Ki67 have been used in an attempt to distinguish odontogenic cysts from ameloblastoma. While differences have shown to occur between various cysts and unicystic ameloblastoma, considerable overlap exists and none of the above techniques can be used to routinely distinguish these

lesions.[4] Recently, Immunohistochemistry with the use of appropriate markers has become popular for differentiation of these pathologic lesions. Several markers like calretinin have been studied in odontogenic lesions to distinguish from each other.[5] Calretinin is a calcium binding protein (CaBp) of 29-kilodalton (kDa) and is a member of the large family of EF hand proteins. EF hand proteins are characterized by a peculiar amino acid sequence that folds up into a helix which acts as the calcium–binding site. Calretinin contains six EF hand stretches. CaBp acts as a mediator of signaling intracellular calcium ions, which are considered to be an important second messenger intervening in several cellular processes, including proliferation and differentiation.[6] and in neurons of sensory pathways**.** Recent investigations have shown that calretinin is also expressed in a wide variety of normal human tissues and in many tumors.[7] The diagnostic sensitivity of calretinin for mesothelioma is near 100%.[8] It is a specific immunohistochemical marker for neoplastic ameloblastic epithelium and may have a role in the transition of the epithelial lining of odontogenic cyst to ameloblastomatous epithelium. Hence, this study was planned to check calretinin as diagnostic tool for differentiating cystic odontogenic lesions from odontogenic tumor.

MATERIALS AND METHODS

The present study was conducted on the archival formalin fixed paraffin embedded tissue blocks of Ameloblastoma, Unicystic ameloblastoma, Dentigerous cyst and Odontogenic keratocyst from the Department of Oral Pathology and Microbiology, Jaipur dental college, Jaipur. A total of eighty cases, in which twenty cases each of Ameloblastoma, Unicystic ameloblastoma, Dentigerous cyst and Odontogenic keratocyst were included in the study. Slides were made from the archival blocks of each case and were stained immunohistochemically with calretinin.

Case history performa was filled from the previously recorded cases. A total of 80 cases were taken and divided into four groups- Group I – comprised of 20 cases of Unicystic Ameloblastoma. Group II – comprised of 20 cases of Dentigerous Cyst. Group III – comprised of 20 cases of Keratocystic Odontogenic Tumor. Group IV – comprised of 20 cases of Ameloblastoma Inclusion criteria- Histologically diagnosed cases of Ameloblastoma, Unicystic Ameloblastoma, Dentigerous cyst and KCOT. Cases with adequate epithelial component.

Exclusion criteria- severely inflamed lesions.

Armamentarium- The material includes Microtome, Autoclave, Hot air oven, Slide warming table, Poly-L-lysine coated microscopic slide, Glass marking pencil, Measuring jar, Disposable masks, Coplin jars, Aluminium foil, Micro-pipettes, Forceps, Slide box and Electronic timer. The sections stained with calretinin antibody and were evaluated for localization, distribution ,and intensity of immunoreactive cells.

RESULTS AND OBSERVATIONS

The software used for the statistical analysis was SPSS (Statistical Package for Social Sciences) version 16.0. The sections stained with calretinin antibody were evaluated for the presence, localization, distribution, and intensity of immunoreactive cells.

Presence - was evaluated to estimate whether the staining is positive or negative and positive which epithelial layer is stained.

Localization - was evaluated whether the staining is in nucleus (N), cytoplasm (C), or both.

Distribution - was evaluated as being either focal (involving < 50% of positive cells) or diffuses (involving > 50% of positive cells).

Intensity - was graded based on the number of positive cells seen

0 – no staining
1 – weak staining
2 – moderate staining
3 – intense staining

Table 1: Correlation of calretinin staining in Histopathologically diagnosed cases

Histopathological Diagnosis	Total no. of cases	Calretinin Staining			
		Positive		Negative	
Ameloblastoma	20	20	100.0%	0	0.0%
Unicystic ameloblastoma	20	17	85.0%	3	15.0%
OKC	20	0	0.0%	20	100.0%
Dentigerous cyst	20	0	0.0%	20	100.0%
Chi-square value = 69.742, p-value < 0.001*					

*** Significant difference**

Table 2- Comparison of calretinin staining in the different Histopathologically diagnosed cases using the Chi-square test

Histopathological Diagnosis	Unicystic ameloblastoma	OKC	Dentigerous cyst
Ameloblastoma	0.072	< 0.001*	< 0.001*
Unicystic ameloblastoma	-	< 0.001*	< 0.001*
OKC	-	-	1.000

The present study assessed the expression of calretinin in 20 cases each of ameloblastoma, unicystic ameloblastoma, dentigerous cyst and odontogenic keratocyst comprising of 62 males and 18 females with the mean age of males being 40.2 years and females 39.7 years. The results demonstrated frequent expression of calretinin in the epithelium of both unicystic ameloblastoma and ameloblastomas, whereas, no case of OKC and dentigerous cyst lining showed positive staining for calretinin. These findings were in accordance with the studies done by **Altini et al. (2000)**[11], **Coleman et al.(2001)**[12] who found positive staining in both unicystic ameloblastoma and ameloblastoma whereas none of the odontogenic cysts lining showed positive staining.

CONCLUSION-

In the present study few nonepithelial cells that stained positive for calretinin were also observed in the connective tissue .the present study suggests that calretin may be used as a Specific immunohistochemical marker for neoplastic epithelium as calretinin positive was observed exclusively in ameloblastomas. Hence, it may serve as an important diagnostic adjunct in the differential diagnosis of ameloblastoma and cystic odontogenic lesions.

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