ORIGINAL RESEARCH

# Evaluation of Calretinin expression in Ameloblastoma and Non-Neoplastic Odontogenic Cysts – An immunohistochemical study

**Shaloom D'Silva[1], M K Sumathi[2], N Balaji[3], Nisha K N Shetty[4], K M Pramod[5], Jacob Cheeramelil[6]**

[1]Private Practitioner, Doha, Qatar; [2]Associate Professor, Department of Oral & Maxillofacial Pathology, Teerthankar Mahaveer Dental College and Research Centre, Teerthankar Mahaveer University, Moradabad, Uttar Pradesh, India; [3]Professor & Head, Department of Oral & Maxillofacial Pathology, Teerthankar Mahaveer Dental College and Research Centre, Teerthankar Mahaveer University, Moradabad, Uttar Pradesh, India; [4]Professor, Department of Oral & Maxillofacial Surgery, Career Dental College & Hospital, Lucknow, Uttar Pradesh, India; [5]Senior Lecturer, Department of Orthodontics, D A P M R V Dental College, Bangalore, Karnataka, India; [6]Senior Lecturer, Department of Orthodontics, Annoor Dental College, Muvattupuzha, Kerala, India.

**ABSTRACT**

**Background:** Calretinin a 29-kDa calcium binding protein is expressed widely in normal human tissue and tumours including amelobastoma. The objective of this study was to determine calretinin expression in heamatoxylin and eosin diagnosed cases of ameloblastoma and non-neoplastic odontogenic cysts.

**Materials & Methods:** The lining epithelium in 3 cases of radicular cysts, 5 cases of odontogenic keratocysts, 5 cases of dentigerous cysts and 11 cases of ameloblastomas were examined for expression of calretinin.

**Results:** No positive epithelial staining was observed in radicular and dentigerous cysts. In comparison, however 100% of cases of ameloblastomas and 40% of cases of odontogenic karatocysts showed positive calretinin expression.

**Conclusion:** Calretinin may be a specific immunohistochemical marker for ameloblastoma. If there is any possible relation between calretinin expression and neural origin of the odontogenic epithelium and its neoplastic transformation and if calretinin could be used as an early marker to predict the tendency of neoplastic change of odontogenic epithelium could be answered through further researches.

**Key Words:** Ameloblastoma, calretinin, odontogenic cysts, odontogenic keratocyst.

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**Address for Correspondence:** Dr. Sumathi M K. Department of Oral & Maxillofacial Pathology, Teerthankar Mahaveer Dental College and Research Centre, Teerthankar Mahaveer University, Moradabad, Uttar Pradesh, India. Phone: +91 – 9444463322. Email: drsumathibalaji@gmail.com

## Introduction

The multiformity of odontogenic tumours reflects the complex development of the normal pattern of odontogenesis.[1] The odontogenic cysts too represent an aberration at some stage of odontogenesis, and in fact may be intimately associated with certain of the odontogenic tumours.[1] Benign but aggressive tumours of the jaws should be differentiated from the non-neoplastic jaw cysts which are well circumscribed. Non-neoplastic odontogenic cysts might show similarities with ameloblastoma and this evoked the need for a specific marker, which would help to differentiate between them. Odontogenic keratocyst is a cyst derived from the remnants of the dental lamina, with a biological behavior similar to a benign

neoplasm. They are unique odontogenic lesions that have the potential to behave aggressively, that can recur and can be associated with the nevoid basal cell carcinoma syndrome.[2,3]

Calretinin is a calcium-binding protein of 29-kilodalton (29 kDa) and is a member of the large family of EF hand proteins.[4] 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 such EF hand stretches. Calretinin is widely expressed in central and peripheral neural tissues,[4-10] particularly in the retina and in neurons of sensory pathways.[11,12] The exact biological function of calretinin remains unknown but possible role as a calcium buffer and regulator of apoptosis have been postulated.[13] Calcium–binding proteins such as calbindin[14,15] and calmodulin[16] have been documented in odontogenic epithelium during tooth development in the rat. A recent, unpublished, study has shown calretinin immune-reactivity in the enamel organ during odontogenesis in the same experimental animal.[13] The pattern of their expression and marked similarities between them with respect to calcium – binding properties could indicate that they perform similar function in neurons. Thus calretinin seems to share with calbindin certain features such as regulation of expression by growth factors and involvement in cell proliferation, differentiation and neoplastic transformation.[17]

**Materials and Methods**

This study was aimed at evaluation of calretinin in non-neoplastic odontogenic cysts and ameloblastomas. The study involved the use of formalin fixed paraffin embedded tissues of previously diagnosed cases of radicular cysts, dentigerous cysts, odontogenic keratocysts and ameloblastomas from our department. Relevant features like, age, sex, site, etc, were obtained from the records of the patients. Normal brain tissue was collected as the positive control. A total of 3 cases of radicular cysts, 3 cases of dentigerous cysts, 5 cases of odontogenic keratocysts, 11 cases of ameloblastomas and 1 case of normal brain tissue were assessed. The following chemicals were used:Anti rabbit polyclonal secondary antibody, Anti calretinin polyclonal primary antibody (Biogenex laboratories Inc., USA), DAB chromogen (3, 3 diaminobenzidine chromogen), Hydrogen Peroxide (0.3%), Phosphate buffer saline (PBS), Sodium citrate buffer (pH 6.0), 2% bovine serum albumin, Xylene, Alcohol (50%, 70% & 100%), Distilled Water, Harris Haematoxylin, Mounting medium (DPX) and coverslips.

Serial sections of 4 um thicknesses were made onto coated slides for immunohistochemistry. The tissue sections were deparaffinized and were then rehydrated. Endogenous hydrogen peroxide in tissue sections was blocked by immersing the slides in PBS containing 0.3% hydrogen peroxide in a staining bath for 30 minutes at room temperature and then antigen was retrieved. After antigen retrieval, the staining bath was placed at room temperature for 20 minutes. The tissue sections were incubated with 3% BSA (blocking agent) and 1% PBS in a humid chamber at room temperature for 30 minutes. After 30 minutes, the excess BSA from tissue sections were removed. The slides were washed by immersing in PBS for 5 minutes at room temperature. The tissue sections were incubated with 100 Ul of polyclonal antibody in refrigerator overnight. The slides were washed by immersing in PBS for 5 minutes at room temperature (Repeat twice). The tissue sections were incubated with 75Ul of rabbit polyclonal secondary antibody at 1:300 dilution in 2% BSA – TBS for 35 minutes at room temperature. The slides were washed by immersing in PBS for 5 minutes at room temperature (Repeat twice). The DAB substrate was prepared just prior to use. The slides were immersed in DAB substrate solution in a staining bath at room temperature for 5 minutes. The tissue sections were counterstained by immersing the slides in heamatoxylin in a staining bath for 2 minutes. The slides were washed in running tap water for 5 minutes. The tissue sections were dehydrated by immersing the slides in increasing concentrations of alcohol (50%, 70%, 100%) in a staining bath for 3 minutes each at room temperature. The slides were immersed in xylene in a staining bath for 6 minutes at room temperature (Repeat twice). The slides were mounted in permanent mounting medium and air dried. The staining was observed under light microscope. Anti – Calretinin stained brown against light blue back ground. The distribution pattern of anti

calretinin was evaluated and analyzed for each group of lesions.

**Results**

The study consisted of brain tissue as positive control and cases of ameloblastomas, dentigerous cysts, odontogenic keratocysts and radicular cysts. The cases were grouped as Group I: Ameloblastoma (11 cases) and Group II: Non Neoplastic Odontogenic Cysts (11 cases). Group II was further divided into, Group IIa : Dentigerous cyst (3 cases), Group IIb: Odontogenic Keratocyst (5 cases) and Group IIc: Radicular Cyst (3 cases).

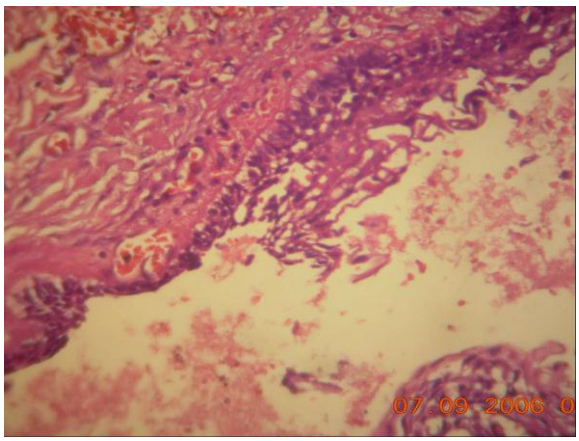

**Figure 1 :** H&E section showing the cystic linig of unicystic ameloblastoma.

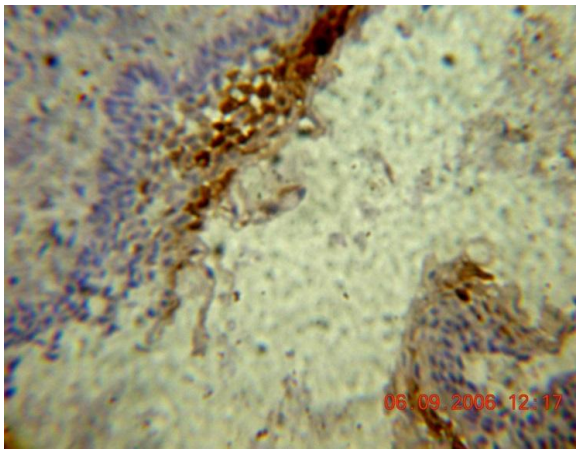

**Figure 2:** Calretinin expression in superficial layers of the lining epithelium of unicystic ameloblastoma.

Calretinin expression in Ameloblastomas: Cases of ameloblastomas included the following clinicopathologic variants; Unicystic, Plexiform, Granular cell, Acanthomatous. Of the 11 cases of ameloblastomas all of them showed positive staining. Expression pattern was clumped, diffuse intense cytoplasmic staining of the superficial and luminal cells, as well as of the stellate reticulum like epithelium in the unicystic variant. Among the solid variants intense staining was noted in the stellate reticulum like cells . Positive staining was noted in the basal cells as well . Areas of squamous metaplasia within the stellate reticulum like epithelium were prominently stained. Cases of granular cell ameloblastomas also showed positive staining of the granular cells. Figure [1-4]

Claretinin expression in Odontogenic Keratocyst: Out of 5 cases of odontogenic keratocysts, 2 cases showed positive calretinin staining of the cystic lining epithelium and keratin flakes in the cystic lumen. Figure [5,6]

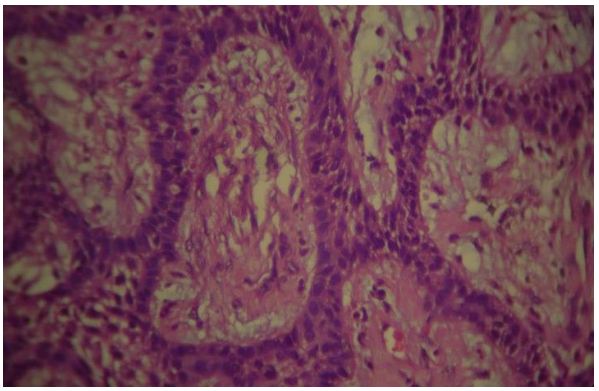

**Figure 3:** H&E section showing anastomosing cords of plexiform ameloblastoma.

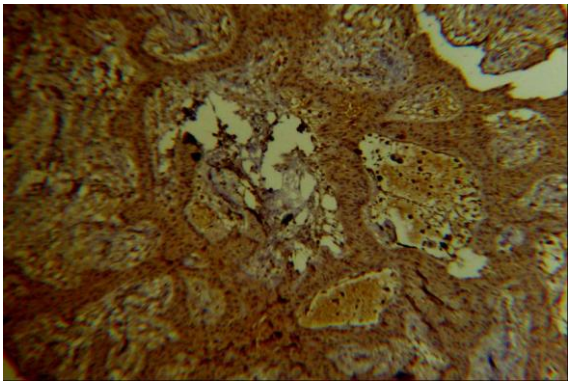

**Figure 4:** Calretinin expression in plexiform ameloblastoma.

Calretinin expression in Dentigerous Cyst: All the cases showed negative staining with calretinin. Figure [7, 8]

Calretinin expression in Radicular Cyst: The epithelium lining the radicular cysts similarly showed no staining except for extravasated RBC's & scattered individual cells with staining of both cytoplasm and nucleus .Table [1-3]

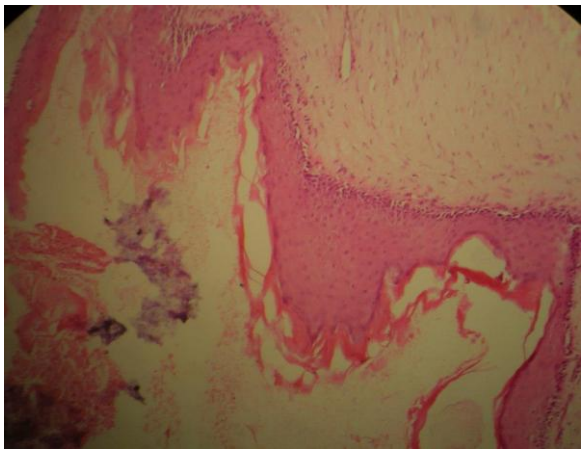

**Figure 5:** H & E section showing corrugated cystic lining of OKC.

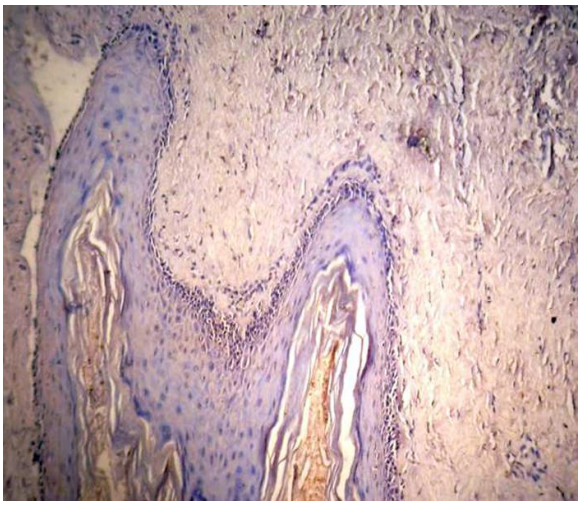

**Figure 6:** Calretinin expression in OKC.

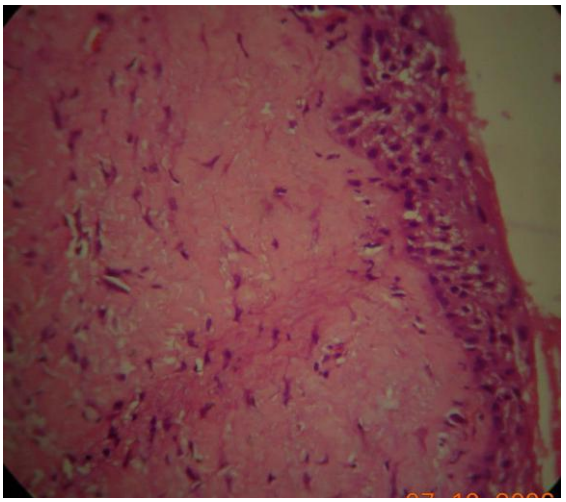

**Figure 7:** H&E showing the thin stratified squamous epithelium of dentigerous cyst.

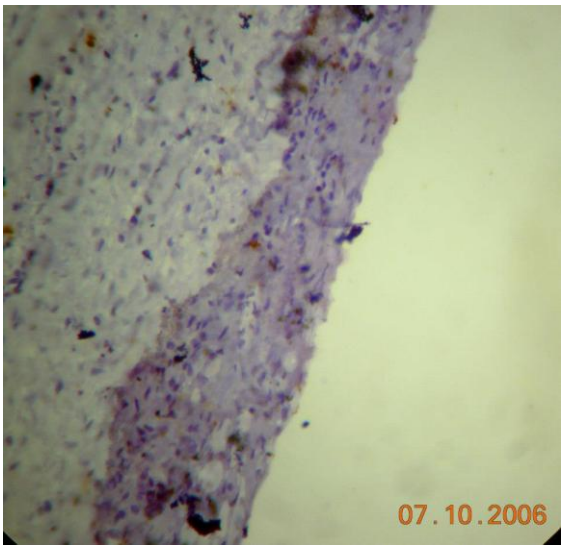


**Figure 8:** Absence of calretinin expression in lining epithelium of dentigerous cyst

## Discussion

Calretinin is widely distributed in many normal and neoplastic human tissues. Its expression in the nervous system has been extensively used by neuro anatomists and it represents by far the most specific and sensitive marker for both benign and malignant mesothelial cells.[13] Calretinin is now established as a marker of neuronal differentiation in central nervous system tumours.[13] Expression of calretinin in many normal human tissues and other human neoplasms have been investigated and its role as a specific immune-histochemical marker has to be elucidated. Only a partial correlation between staining of normal cells and their neoplastic counterparts has been observed. Calretinin expression has been demonstrated in the

**Table 1: Calretinin expression in Group I and Group II**

| | N | Positive | Negative | P.Value |
|---|---|---|---|---|
| Group I | 11 | 11 | 0 | 0.000 |
| Group II | 11 | 2 | 9 | |

Table 1 shows calretinin expression in Group I and Group II which showed a positivity of 100% and 18.2% of cases respectively.

neural elements of the tooth pulp, periodontal ligament[10,18] and in viscerosensory nerve fibers in oral and pharyngeal tissues in the rat.[13] Calcium binding proteins have been demonstrated in the odontogenic epithelium during odontogenesis in tooth germs.[13,19,20] Calretinin was expressed focally in dental lamina, outer enamel epithelium, stellate reticulum and stratum intermedium at different stages. In contrast it was more diffusely and intensely stained in the inner enamel epithelium secretory ameloblasts; in the secretory ameloblast the staining was less intense, being restricted to the cytoplasm. Localization may suggest that calretinin may play a role in differentiation.[20] Many techniques have been used in an attempt to distinguish odontogenic cysts from unicystic ameloblastoma. They included: demonstration of cell surface carbohydrate with blood group specificity;[21] determination of alkaline phosphatase activity in the stroma;[22] distribution of lectins and involucrin in the epithelium[23] characterization of cytokeratin profile;[24] counting of AgNORs[25] and quantification of cell proliferation markers such as PCNA and Ki 67.[26]

While differences have been shown to occur between the various cysts and the unicystic ameloblastoma, considerate overlap exists between them and none of the above techniques can be used to routinely distinguish these lesions from one another.

**Table 2: Calretinin expression in different groups**

| | N | Positive | Negative | P.Value |
|---|---|---|---|---|
| Group I | 11 | 11 | 0 | 0.001 |
| Group IIa | 3 | 0 | 3 | |
| Group IIb | 5 | 2 | 3 | |
| Group IIc | 3 | 0 | 3 | |

Table 2 shows calretinin expression in different groups. Calretinin expression in the study groups comprising of I, IIa, IIb, IIc showed positivity in 100%, 0%, 40% and 0% of cases respectively .

**Table 3: Comparison of calretinin expression between Group I and Group IIa, Group I and Group IIb , Group I and Group IIc**

| | N | Positive | Negative | P. Value |
|---|---|---|---|---|
| Group I | 11 | 11 | 0 | 0.01 |
| Group IIa | 3 | 0 | 3 | |
| Group I | 11 | 11 | 0 | 0.05 |
| Group IIb | 5 | 2 | 3 | |
| Group I | 11 | 11 | 0 | 0.01 |
| Group IIc | 3 | 0 | 3 | |

Table 3 shows comparison of calretinin expression between Group I and Group IIa, Group I and Group IIb, Group I and Group IIc Comparison between each group were statistically significant.

The present study was carried out mainly to evaluate calretinin expression in various non-neoplastic odontogenic cysts (i.e. odontogenic keratocyst, dentigerous cyst and radicular cyst) and odontogenic tumour (ameloblastoma). The current study showed 100% expressivity in ameloblastomas, 0% expressivity in radicular cysts, 0% expressivity in dentigerous cysts and 40% expressivity in odontogenic keratocyst. The 100% positivity in cases of ameloblastoma can be explained on the basis of the study done by Coleman HG et al[13] that calretinin is expressed in the odontogenic epithelium of tooth germs at various stages of development.

The present study as the previous study by H Coleman et al[16] showed both areas of non-descript epithelial linining and areas with ameloblastic features with

positive staining. This indicates that although the metaplastic cyst linings may have lost their typical ameloblastic features, the cells have retained their immunophenotypic characteristics resulting in the continued expression of calretinin. The 40% positivity in the odontogenic keratocyst were seen in the orthokeratinized epithelium, this was in contrast to the previous study by H. Coleman[17] which showed absence of staining in any of the cases. Our finding could be explained as odontogenic keratocyst is a cyst derived from remnants of the dental lamina, with biologic behaviour similar to a benign neoplasm. They are unique odontogenic lesions that have the potential to behave aggressively, that can recur, and can be associated with nevoid basal cell carcinoma syndrome. Main (1970a) and Toller (1971), showed that mitotic value of keratocyst linings ranged from 0 to 19 which was similar to that in the ameloblastoma and in dental lamina, and higher than that found in non-odontogenic cysts which has a mean mitotic value of 2.3.[27] Scharfetter et al (1989),[28] stated that the epithelium of the keratocyst showed a higher rate of proliferation than the radicular cyst and positivity could be related to the increased mitotic value, as studies by Gotzoz et al (1996) showed that calretinin was found in rapidly proliferating cells.[17] Toller (1967), suggested that OKC's might be regarded as a benign cystic neoplasms. Whether they are developmental or neoplastic continues to be debated.[1] Correlating the expression of calretinin in nervous system, the odontogenic epithelium of tooth germs and in neoplasms of odontogenic epithelial cells possibly might be due to their derivation from neural crest cells.

**Conclusion**

In the study groups calretinin expression showed a positivity in 100% and 18.2% of cases of Groups I & II respectively. Groups I, IIa, IIb and IIc showed calretinin expression in 100%, 0%, 40% & 0% of cases. Calretinin expression between Groups I & IIa, I & IIb, I & IIc were statistically significant. From the results obtained in the current study it may be concluded that, Calretinin expression is noticed in odontogenic neoplasm ie., ameloblastoma at varying degrees of intensity. Odontogenic Keratocyst showed positive calretinin expression when they had a more aggressive potential compared to their counterparts.Radicular Cysts and Dentigerous Cysts do not express calretinin. Calretinin has several avenues of potential impact since varying levels of calretinin expression was noted in all cases of ameloblastomas and 40% of OKCs. With this study we suggest that calretinin may be a specific immunohistochemical marker for ameloblastoma.If there is any possible relation between calretinin expression and neural origin of the odontogenic epithelium and its neoplastic transformation and if calretinin could be used as an early marker to predict the tendency of neoplastic change of odontogenic epithelium could be answered through further researches.

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