Original Article

Calretinin expression in odontogenic cysts and odontogenic tumors and the possible role of calretinin in pathogenesis of ameloblastoma

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Abstract

Introduction: Histopathological diagnosis of odontogenic cysts and tumors is a task mostly accomplished with hematoxylin and eosin staining. However, the use of additional diagnostic modalities such as immunohistochemistry may be necessary in histologically similar lesions. The reports of studies which have used calretinin as an immunohistochemical marker for ameloblastoma have been conflicting.

Aim: The aim of the study was to evaluate the use of calretinin as a specific diagnostic marker for ameloblastoma and observe its expression in odontogenic cysts and other odontogenic tumors.

Materials and Methods: Formalin-fixed, paraffin-embedded sections were taken from the archives which included 15 cases each of dentigerous cyst, radicular cyst, odontogenic keratocyst and ameloblastoma five cases of adenomatoid odontogenic tumor and three cases of ameloblastic carcinoma. Immunohistochemistry was done with calretinin antibody.

Results: All ameloblastomas were positive for calretinin, whereas no other tumor or cyst showed positivity. Differences in proportion of calretinin expression were statistically significant with $P = 0.000$.

Conclusion: Calretinin can be considered as a specific marker for ameloblastomas.

Keywords: Ameloblastoma, calretinin, odontogenic cyst, odontogenic tumor

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INTRODUCTION

Odontogenic cysts and odontogenic tumors are a diverse group of lesions. They are of epithelial, ectomesenchymal and/or mesenchymal origin and exhibit varying degrees of inductive tissue interactions.[1]

Accurate diagnosis of these lesions in most cases may be arrived at, with clinical and radiographic examination and histopathological findings of hematoxylin and eosin-stained biopsy specimens. Many benign jaw tumors and several cysts of odontogenic origin can exhibit a biologically

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aggressive course, often mimicking malignancies and sometimes making diagnosis difficult. Variable appearance of the same lesion and overlapping histology among different lesions may lead to further difficulties. However, appropriate diagnoses are crucial as their treatment modalities and prognoses differ. In such situations, more specific techniques might be needed to aid in the diagnosis, and to avoid overdiagnosis or underdiagnosis, leading to inefficient treatment.

Calretinin, a 29 kDa calcium-binding protein, primarily expressed in certain subtypes of neurons, has been shown to be positive in the odontogenic epithelium during different stages of odontogenesis.[2-4] The same has been documented in some odontogenic cysts and tumors, and few studies have shown it to be a specific marker for ameloblastoma.[5,6] However, all studies do not agree with this view, and they have shown that calretinin is not specific for ameloblastoma.[7,8]

The purpose of this study, therefore, was to evaluate the use of calretinin as a specific diagnostic marker for ameloblastoma by comparing its expression in common odontogenic cysts and other odontogenic tumors which are likely to confound the diagnosis.

MATERIALS AND METHODS

A total of 68 formalin-fixed, paraffin-embedded sections were taken from the archives of the department of oral pathology. They included 15 cases each of dentigerous cyst, radicular cyst, odontogenic keratocyst (OKC) and ameloblastoma (7 unicystic and 8 solid/multicystic), five cases of adenomatoid odontogenic tumor (AOT) and 3 cases of ameloblastic carcinoma. The previously established diagnoses of the selected cases were confirmed according to the WHO criteria.[9]

Sections of 4-μm thickness were taken using a semi-automatic rotary microtome (Microm HM340E, Germany). The sections were taken on positively charged microscopic slides (Fisher Scientific). Antigen retrieval was done using BioGenex antigen retrieval system (E7 Retriever system V.2.2) and antigen retrieval buffer with pH: 8.5–9.0 (1.21 g tris buffer + 0.37 g disodium EDTA). The heat cycles were as follows: Cycle 1: buffer at 95°C for 5 min; Cycle 2: buffer and slides at 98°C for 10 min; Cycle 3: buffer and slides at 98°C for 5 min and Cycle 4: buffer and slides at 98°C for 5 min.

Immunohistochemistry was done with the primary antibody, calretinin monoclonal mouse antihuman calretinin (DAK-Calret1, Dako). The findings were then observed under a trinocular research microscope (Labomed, India), and digital images were captured with a photomicrographic camera (Jenoptik Progres C14 plus) and evaluated. Brown color staining, both nuclear and cytoplasmic, was considered as positive staining and expression of calretinin.

Quantitative and qualitative analyses were performed for the sections which were confirmed positive for calretinin. Intensity, pattern of distribution and localization of the immunoreactive cells were determined using the conventional light microscopy. All the analyses for the above criteria were done under ×200 for three randomly selected fields. In case of follicular ameloblastoma, if the field lacked a follicle, then another field was chosen.

All the positive cells in each field were given scoring as 0 – no staining, 1 – mild staining, 2 – moderate staining and 3 – intense staining. Then, the total number of cells for each score was calculated for three fields and percentage was calculated. The score having a maximum percentage of cells was considered as the intensity of the slide analyzed.

The pattern of distribution was assessed as diffuse and focal. This was determined by using the following formula:

$$P\% = \frac{\text{Number of positive cells}}{\text{Total number of cells in a field}} \times 100$$

Average of three fields was taken for each specimen, and if *P*% was ≥50, the specimen was graded as diffuse, and when *P*% was <50, it was graded focal.

Finally, localization of the positive cells was assessed, as whether they were confined to stellate reticulum-like cells or ameloblast-like cells in ameloblastomas.

The Chi-square test was used to statistically compare the expression of calretinin among different cases. $P < 0.05$ was considered statistically significant.

RESULTS AND OBSERVATIONS

All the ameloblastomas included in the study were positive for calretinin, and the positivity was confined to the odontogenic epithelium. No other tumors and cysts showed positivity for calretinin [Figures 1, 2 and Graph 1].

Of the seven unicystic ameloblastomas, five cases were diffusely positive and two were focally positive. Intensity of staining was moderate in two cases and five were intensely positive. Staining was confined to stellate reticulum-like cells, sparing the most superficial cells as well as the basal columnar

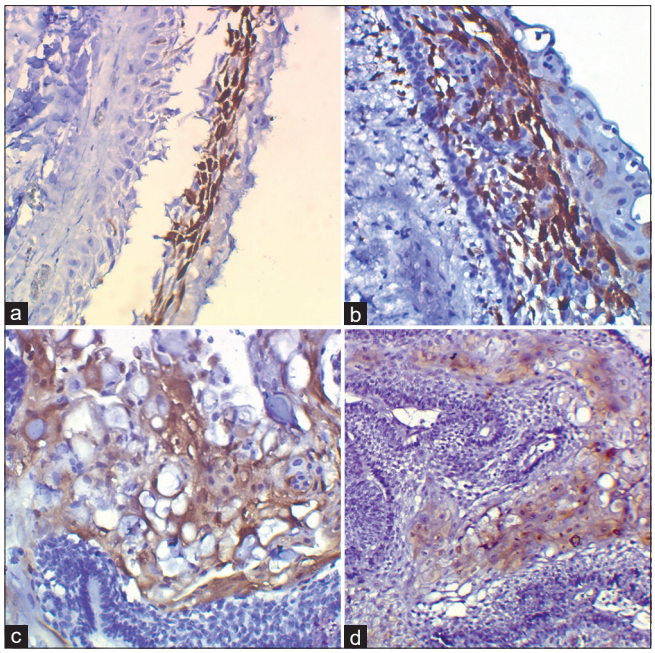


Figure 1: Photomicrograph of unicystic ameloblastoma (a and b), follicular ameloblastoma (c) and plexiform ameloblastoma (d) showing immunoreactivity to calretinin (×200)

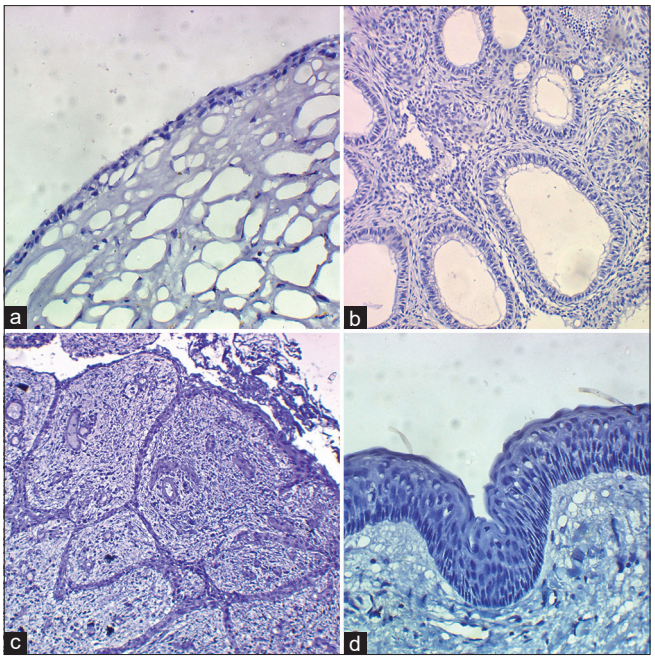


Figure 2: Photomicrograph of dentigerous cyst (a), adenomatoid odontogenic tumor (b), radicular cyst (c), odontogenic keratocyst (d), showing no immunoreactivity to calretinin (×200)

cells, producing a band-like pattern. Immunoreactivity was not continuous throughout the length of the epithelium. In the areas predominated by nondescript epithelium, the staining was positive but was not as intense and continuous as observed in areas predominated by the epithelium with typical ameloblastic features [Figure 1].

In case of solid/multicystic ameloblastoma, all five cases of follicular ameloblastoma were diffusely positive. Two cases of plexiform variant showed focal positivity and one was diffusely positive. In the follicular variant, four cases were intensely positive with moderate intensity in one case, and in the plexiform variant, two were intensely positive and one case showed moderate intensity.

In all the cases, staining was restricted to the stellate reticulum-like areas. None of the peripheral ameloblast-like cells were immunopositive. Wherever there was macrocyst or microcyst formation within the epithelial islands, the cells lining the cysts stained more prominently. However, the staining was not equally distributed throughout the section, with some areas of intense positivity being interspersed with those showing absolutely no staining [Figure 1].

In all the ameloblastomas, scattered positive cells were seen within the stroma, which were thought to be nonepithelial in origin.

In case of ameloblastic carcinoma, no reactivity was seen in the epithelial islands, neither in the basal cells nor in the cells in the central portion of the island. However, numerous positive cells, similar in appearance to the cells in the connective tissue of other lesions, were concentrated adjacent to the neoplastic epithelial islands [Figure 2].

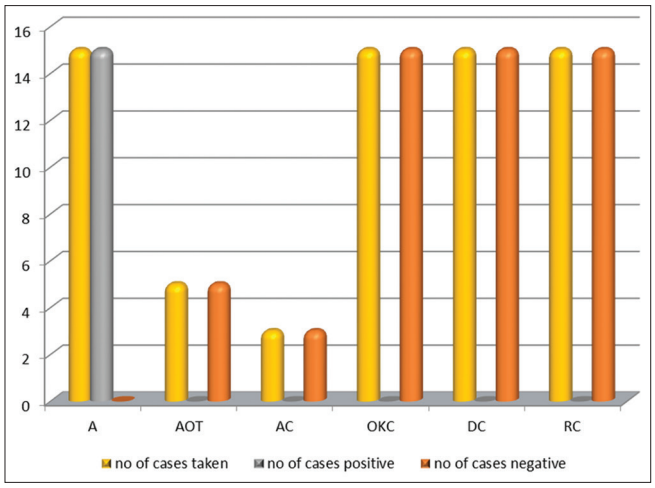
All cases of AOT, dentigerous, radicular and OKCs showed no reactivity for the calretinin, except for the scattered positive cells observed in the connective tissue, which were interpreted as mast cells. Adipocytes present in the connective tissue of some lesions also showed positivity for calretinin [Figure 2].

Differences in proportion of calretinin expression were statistically significant with $P = 0.000$ [Table 1].

DISCUSSION

The jaws are host to a wide variety of cysts and neoplasms. Most of these are of odontogenic origin, due in large part to the tissues involved in tooth formation. Although the diagnosis of ameloblastoma is obvious in most occasions, sometimes, the diagnosis is challenging due to heterogeneity in histology and variety of histologic mimics.

For instance, pseudoepitheliomatous hyperplasia merging with the ameloblastic tumor and acanthomatous variant make the distinction from squamous cell carcinoma difficult, which is essential to avoid overtreatment.[10] Similarly, distinguishing from benign lesions is vital in the view of the locally aggressive nature of ameloblastoma



Graph 1: Depicting the expression of calretinin in odontogenic cysts and tumors. A: Ameloblastoma, AOT: Adenomatoid odontogenic tumor, AC: Ameloblastic carcinoma, OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 1: Expression of calretinin in odontogenic cysts and tumors and intercomparison among different groups using the Chi-square test

Lesion	Number of cases positive	Number of cases negative	Total	Test value	*P*
A	15	0	15	68.000	0.000
AOT	0	5	5		
AC	0	3	3		
DC	0	15	15		
OKC	0	15	15		
RC	0	15	15		

A: Ameloblastoma, AOT: Adenomatoid odontogenic tumor, AC: Ameloblastic carcinoma, OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

which necessitates radical excision. In unicystic ameloblastomas, with predominantly nondescript epithelial lining, distinguishing from radicular and residual cysts can be intriguing. OKC may exhibit spongiosis, resembling closely the stellate reticulum-like cells. Small tissue sample or in cases where lesional epithelium displays reactive changes induced by inflammation, it can closely resemble unicystic ameloblastoma histologically. Overlapping clinical and radiographic presentation further adds to this diagnostic difficulty. Immunohistochemical evaluation plays an important role in such cases.

Calretinin, a calcium-binding protein, has a widespread distribution in many normal and neoplastic tissues, as well as in odontogenic epithelium during odontogenesis.[2-4,11,12] A few studies, hence, were conducted to evaluate its expression in odontogenic cysts and tumors.[5-8,13-16] Although some of these studies have shown calretinin to be a marker for ameloblastoma, a few have also shown its expression in OKCs.[7,8]

Since there are very few studies on calretinin expression in odontogenic cysts and tumors and due to the variability in the reported results, we intended to perform the present study. As the aim of the present study was to evaluate calretinin as a specific marker for ameloblastomas, other odontogenic tumors and cysts were also included, depending on their availability in the department archives.

In the present study, positive staining of the odontogenic epithelium was observed only in ameloblastomas. Positivity was 100% for unicystic ameloblastomas, which was in accordance with studies of DeVilliers *et al.*, Sundaragiri *et al.* and D'Silva *et al.*[7,14,15] Altini *et al.* showed that positive staining in 81.5% of unicystic ameloblastomas and the epithelial lining in the negative cases were intensely inflamed and showed squamous metaplasia and arcading and one case with typical ameloblastic epithelium was negative.[6]

Anandani *et al.* observed 50% positivity in unicystic ameloblastomas.[16] They attributed the absence of staining to better differentiation of the epithelium as reported by Altini *et al.*[6] However, Altini *et al.* reported that this was not a consistent feature, and in the subsequent report of findings of the same study, they reported uniform staining of both stellate reticulum-like cells and the nondescript epithelium.[5,6]

Staining intensity and distribution pattern of unicystic ameloblastoma, observed in the present study, were in accordance with the previous studies.[6,7,14-16] Immunopositivity was confined to the stellate reticulum-like cells in unicystic ameloblastomas. No staining of the basal ameloblast-like cells was observed. Even when the epithelium was of nondescript type, positivity could still be appreciated. It is likely that metaplastic cyst linings still retain their immunophenotype.[5] Similar spatial distribution was observed in all the reported studies, except for D'Silva *et al.* who reported positivity in the basal cells as well.[6,7,14-16]

In solid/multicystic ameloblastomas, 100% positivity was observed; the distribution pattern and intensity of positivity were correlating with the previous studies.[6,7,13-16] Immunopositivity was confined to stellate reticulum-like cells, with prominent staining around microcysts and macrocysts. The peripheral ameloblast-like cells were negative. These findings were similar to the observations reported previously.[5,6,8,13-16] However, focal positivity of basal cells was reported in one case by Altini *et al.*, in one case by Anandani *et al.,* and in some cases by DeVilliers *et al.*[6,14,16] In contrary, D'Silva *et al.* reported the positivity in peripheral ameloblast-like cells in all their cases.[7]

In the enamel organ of developing tooth, calretinin expression in the stellate reticulum-like cells is reported to be moderate-to-intense and nonuniform during the

early and late bell stages of tooth development which is similar to that observed in the stellate reticulum-like cells in ameloblastomas.[3] The lack of uniformity in staining intensity could be due to variable expression of calretinin, which is governed by the metabolic activity of the cells.[6] It could also be related to impaired calcium homeostasis associated with cellular degeneration as observed around the areas of cystic degeneration.[17]

Expression of calretinin in peripheral ameloblast-like cells was different from that during normal odontogenesis.[3] The inner enamel epithelial cells do not attain functional maturation as secretory ameloblasts in ameloblastoma, which has been attributed to the mutation of ameloblastin.[18] In high probability, calretinin might also have a similar role in cytodifferentiation of inner enamel epithelium (IEE) cells, which is evidenced by the fact that calretinin is absent in the inner enamel epithelium of rat molar cusp tips, where the enamel is not normally present in mature teeth [Figure 3].[3]

Mutations in calretinin gene might have occurred and evidenced by the expression of significant levels of calretinin gene at the mRNA level in ameloblast-like cells of ameloblastoma but lack of its protein.[14] This might be responsible for lack of functional maturation. In addition, it can result in an imbalance in the calcium levels which can cause further genetic imbalance in the cells, thereby having a role in tumorigenesis [Figure 4].[19,20]

No calretinin expression was observed in the other odontogenic tumors, and similar findings were reported by Alaeddini *et al.* who included AOTs, ameloblastic fibromas and odontogenic myxomas in their study.[13] Lack of calretinin expression in AOT might be due to its origin from postsecretory ameloblasts or reduced enamel epithelium.[13]

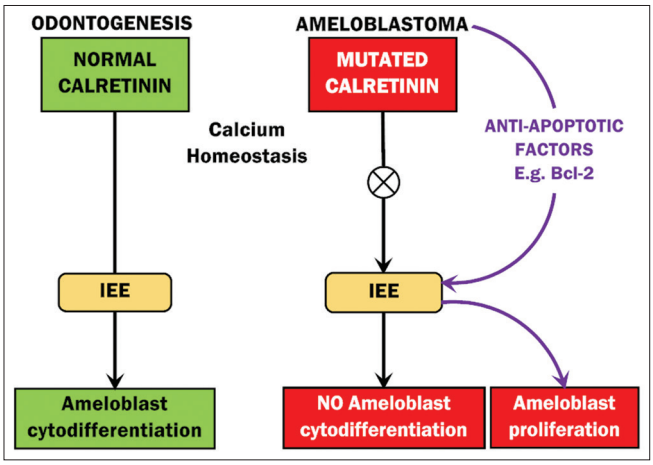


Figure 3: Role of calretinin in odontogenesis and ameloblastoma

Most odontogenic tumors originate from successional and accessional dental laminae but produce a wide spectrum of tumors. Various cellular, subcellular and developmentally related factors and mechanisms that trigger the proliferation of odontogenic epithelial rests may be responsible for the differences in behavior and presentation of these tumors. Calretinin may have a role in the transition of dental lamina remnants to ameloblastoma and might be among the factors responsible for the aggressiveness of ameloblastoma, a feature not observed in other odontogenic tumors.[5]

In ameloblastic carcinomas, neoplastic epithelial islands were negative for calretinin, and there are no previous studies related. Numerous positive cells were observed in the connective tissue and were interpreted as mast cells.

None of the OKCs were positive. This was in accordance with the studies of Coleman *et al.*, DeVilliers *et al.* and Sundaragiri *et al.*[5,14,15] Anandani *et al.* reported positivity in one case which, according to them, might be a misdiagnosed keratocystic odontogenic tumor (KCOT) as the specimen revealed highly inflamed cystic epithelium.[16] A similar experience was reported by DeVilliers *et al.,* wherein they evaluated a tumor and its recurrence from their archives. Histological sections from the earlier biopsy (diagnosed with KCOT) and those from the recurrence (diagnosed with ameloblastoma) were tested with calretinin and both showed positive staining of the neoplastic epithelium.[14] Later, they diagnosed the earlier case to be misdiagnosed KCOT. This finding helps us to understand the importance of calretinin as a diagnostic marker to distinguish between KCOT and ameloblastoma. D'Silva *et al.* showed 40% of OKCs to be positive for calretinin expression. Piattelli *et al.* also showed 8 of the 12 parakeratinized cysts to be positive for calretinin, confined to the parabasal layers; however, orthokeratinized keratocysts were negative.[7,8] Koneru *et al.* also showed 24 of the 30 parakeratinized OKCs to be positive for calretinin, but orthokeratinized cysts were not included in the study.[21] Negative expression, however, does not rule out the involvement of calretinin in the pathogenesis of OKC. There might be aberrant expression of calretinin gene even in OKCs, but the extent to which the gene is mutated might be variable which could be the reason for the variation of results reported in different studies.

All the dentigerous and radicular cysts in the present study were negative for calretinin, like earlier studies.[5,7,8,15,22] These lesions originate from different odontogenic epithelia; like dentigerous cysts from the reduced enamel epithelium and radicular cysts from the cell rests of Malassez and

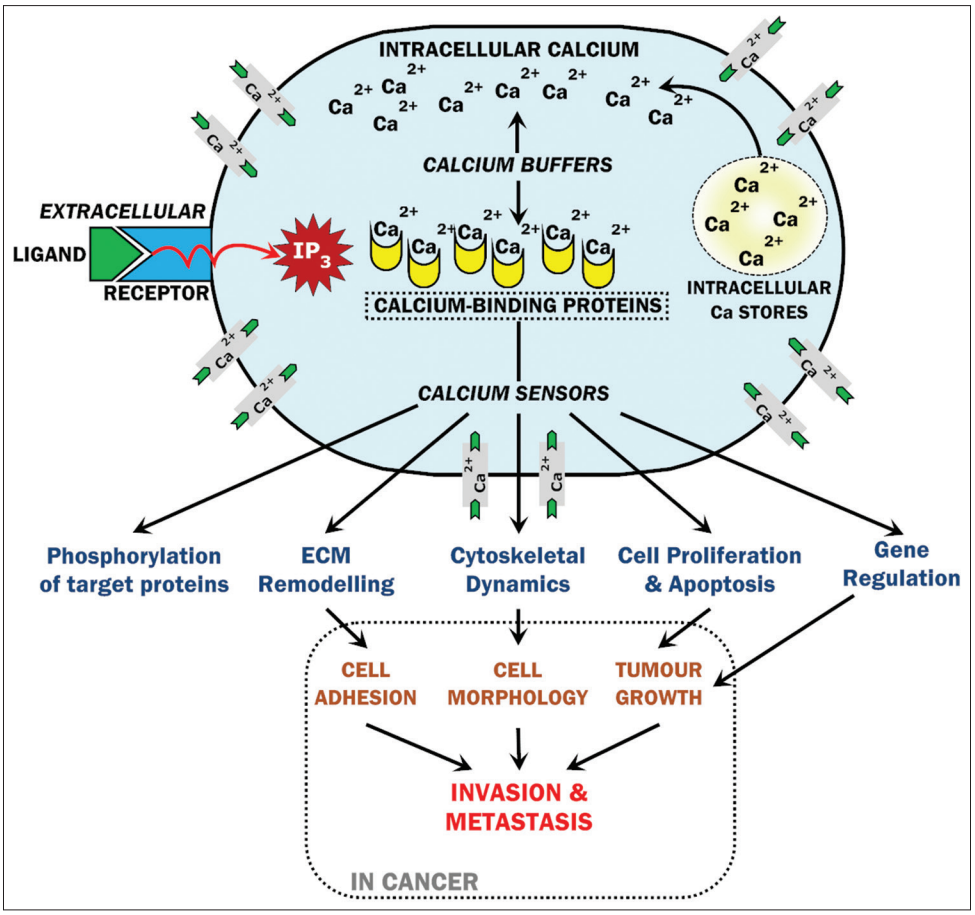


Figure 4: Role of calcium-binding proteins in normal and neoplastic cells

the expression of calretinin might be recapitulating dental ontogeny.[5]

CONCLUSION

Calretinin can, hence, be considered as a specific marker for ameloblastomas. Negative expression in ameloblast-like cells might be related to its mutation, leading to lack of cytodifferentiation and may also have a role in the aggressive behavior of ameloblastoma. The absence of calretinin in OKCs has to be further analyzed as having a role in its pathogenesis. Limitation of the present study was that cases of other odontogenic tumors such as ameloblastic fibromas and odontogenic myxomas were not included; ameloblastic carcinomas, though included, were very few be able to conclude. Additional immunohistochemical and molecular studies are required to substantiate these findings. Such studies can help in using this protein as a therapeutic modality too.

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Conflicts of interest
There are no conflicts of interest.

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