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COMPARATIVE STUDY OF CALRETININ EXPRESSION IN ODONTOGENIC CYSTS AND TUMORS

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ABSTRACT

Calretinin is a calcium-binding protein with molecular weight of 29 kDa (KiloDalton). It is expressed in a variety of normal and tumorigenic tissues. Its expression in odontogenic epithelium during odontogenesis and in neoplastic odontogenic tissues has been demonstrated. This study was undertaken to evaluate the expression of Calretinin in a group of odontogenic cysts and tumors. The expression of Calretinin was evaluated immunohistochemically in 42 samples including 8 cases of periapical cyst, 7 cases of dentigerous cyst, 7 cases of keratocystic odontogenic tumor, 8 cases of solid (multicystic) ameloblastoma, 6 cases of unicystic ameloblastoma and 6 cases of adenomatoid odontogenic tumor. Positive immunohistochemical reaction was found in all cases of solid ameloblastoma (100%), in 4 cases of unicystic ameloblastoma (67%) and 4 cases of keratocystic odontogenic tumors (57%) whereas none of the other odontogenic cysts and adenomatoid odontogenic tumors included in this study showed reactivity. Intensity was higher in the ameloblastomas compared with the keratocystic odontogenic tumors. On the basis of these results, it is suggested that calretinin might be used as a specific immunohistochemical marker for the ameloblastomas and could play an important role in the differentiation of aggressiveness of different odontogenic tumors.

KEY WORDS: Odontogenic cyst and tumors, Calretinin, Immunohistochemistry

INTRODUCTION

Odontogenic cysts and tumors are a group of lesions arising from the tooth-producing apparatus or its remnants. They may originate from odontogenic epithelium and / or ectomesenchyme with varying degrees of inductive tissue interaction.[1] They have a wide spectrum of histopathologic and clinical characteristics either benign or malignant lesions. Histopathologic similarities between these lesions may challenge the correct diagnosis, especially in small size specimens and may also lead to unnecessary broad surgery.[2- 6] Radicular, dentigerous, and odontogenic keratocyst (OKC) are the most commonly occurring odontogenic cysts.[7] The biological behavior of few OKCs is as aggressive as benign neoplasm.

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The clinically aggressive behavior is a result of the properties of lining epithelial cells and connective tissue capsule.[8] OKC is now designated by the World Health Organization (WHO) as a benign tumor of odontogenic epithelium called keratocystic odontogenic tumor (KCOT) to emphasize its neoplastic nature.[9]

Unicystic ameloblastoma is a variant of ameloblastoma in which there is ameloblastomatous development in a pre-existing cyst. It is well known to be lined by a variable epithelium ranging from one that has typical ameloblastic characteristics to one that is metaplastic and which appears completely nondescript consisting of several layers of nonkeratinizing squamous cells. In such cases, the differentiation of odontogenic cysts from the unicystic ameloblastoma can be problematic.[10] The histologic presentation of ameloblastoma, especially unicystic type, can be, in some instances, mistaken for keratocystic odontogenic tumor (KCOT). Overlapping clinical and radiographic presentation further adds to this diagnostic difficulty.[11]

Calcium ions regulate a large number of biological processes such as metabolism, contraction, secretion, cell division, cell growth, and memory storage either directly or indirectly. Elevated cytoplasmic calcium levels found in several tumor cells might contribute to the increased motility and hence invasiveness of these cells. The calcium signal is transmitted into the intracellular response via interactions with a wide variety of intracellular calcium-binding proteins that are involved in the regulation of many cellular activities. One class of these proteins shares a common calcium-binding motif is the EF-hand protein.[12]

Calretinin is a calcium binding protein of 29 kDa (KiloDalton), it is a member of the EF-hand proteins. This protein is expressed primarily in certain subtypes of neurons in the retina and in the central and peripheral nervous system. Its precise behavior is still unknown but possible roles are calcium buffer, calcium sensor, and regulator of apoptosis.[13] Studies in rats have demonstrated calretinin expression in neural elements of the tooth pulp, periodontal ligament and nerve fibers of oral and pharyngeal tissues, as well as in odontogenic epithelium during odontogenesis in molar tooth germs.[14] Further assessment of molecular aspects and immunohistochemical markers such as calretinin in normal and neoplastic tissues may provide a better understanding of the biological behaviour, influencing factors and tumorigenesis of neoplasms including odontogenic tumor.[11]

The aim of the present study is to assess the expression of calretinin in odontogenic cysts and tumors in order to gain a better understanding of the nature of these lesions and the possible role of calretinin in their pathogenesis.

MATERIALS AND METHODS

This study comprised 42 paraffin-embedded tissue blocks including 8 cases of periapical cyst, 7 cases of dentigerous cyst, 7 cases of keratocystic odontogenic tumor, 8 cases of solid (multicystic) ameloblastoma, 6 cases of unicystic ameloblastoma and 6 cases of adenomatoid odontogenic tumor. These blocks were collected from the stored blocks of Pathology Department, National Cancer Institute, Cairo University and Oral Pathology Department, Faculty of Dental Medicine, Al-Azhar University (Assiut branch). The specimens were cut at 5µm thickness for H&E staining as a routine stain and immunohistochemical staining.

Immunohistchemistry:

The sections were mounted on silicon-coated glass slides, deparaffinized in xylene and rehydrated with a descending series of ethanol. After blocking endogenous peroxidase activity with methanol containing 0.3% H_2O_2 for 30 min, antigenicity was retrieved by microwave heating for a period of 5 min (2-3 times) in a citrate buffer (pH 6), then non-specific staining blocking reagent for 10 min to block nonspecific staining. The sections were incubated with anti-calretinin polyclonal rabbit

antibody (Biogenex Life Sciences, CA, USA) at 4ºC overnight at dilution 1:100. After rinsing with phosphate buffer saline (PBS), secondary biotinylated antibody was then applied followed by incubation with streptavidin peroxidase for 30 minutes. To develop brown reaction, the sections were stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and washed with PBS. The slides were counterstained with Mayor's hematoxylin and washed in running tap water. Finally the slides were dehydrated in ascending grades of alcohol, mounted and covered with cover slips. Omission of primary antibody was employed as negative control, colon tissue was used as positive control.

RESULTS

In all immunopositive cases reactivity was observed in both nucleus and cytoplasm. In the solid (multicystic) ameloblastoma, which is composed of nests or islands of tumor cells with stellate reticulum like tissue centrally and ameloblast-like cells peripherally (Figure 1a), there was immunopositive reaction for Calretinin in all cases (100%), Immunoreactivity was restricted to the stellate reticulum-like cells. None of the peripheral ameloblast-like cells were immunoreactive for calretinin. Some cases showed prominent reactivity particularly in areas of squamous metaplasia within the stellate reticulum-like cells and those cells lining cystic degeneration (macrocysts and microcysts) (Figure 1b). In Unicystic Ameloblastoma, there was ameloblastomatous development in a pre-existing cyst (Figure 2a), out of the 6 cases of unicystic ameloblastoma positive calretinin expression of the ameloblastic epithelium was seen in 4 cases (67%). 2 cases showed weak staining, 1 showed moderate, and 1 showed intense staining. In all the immunopositive cases, reactivity was limited to the stellate reticulum like area. The luminal layer of ameloblast-like cells did not stain, except for the few single basal cells that were positive in some cases (Figure 2b). In keratocystic odontogenic tumor, which 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 and the acanthomatous differentiation of ameloblastoma (Figure 3a), 4 out of 7 cases (57%) were immunopositive for calretinin. Of these, 1 case showed moderate staining in the basal and intermediate layers of the cystic epithelium and 3 cases showed mild staining in all cell layers (Figure 3b). All cases of periapical cyst, dentigerous cyst, and adenomatoid odontogenic tumor were completely negative for calretinin staining. These results were summarized in table 1.

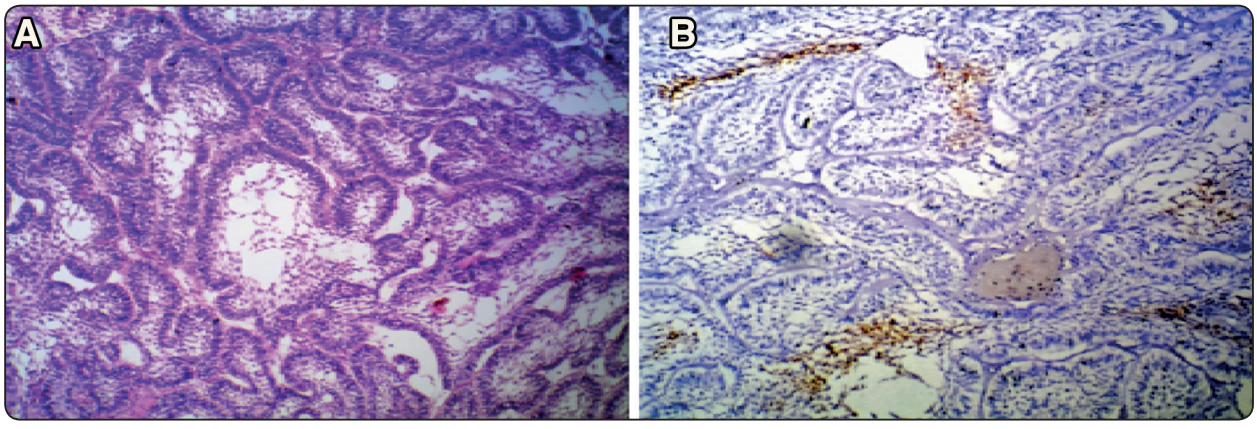

Fig. (1) H&E section showing solid ameloblastoma (A) (X200), Calretinin expression in solid ameloblastoma showing moderate staining reaction in stellate reticulum-like cells (B) (X200)

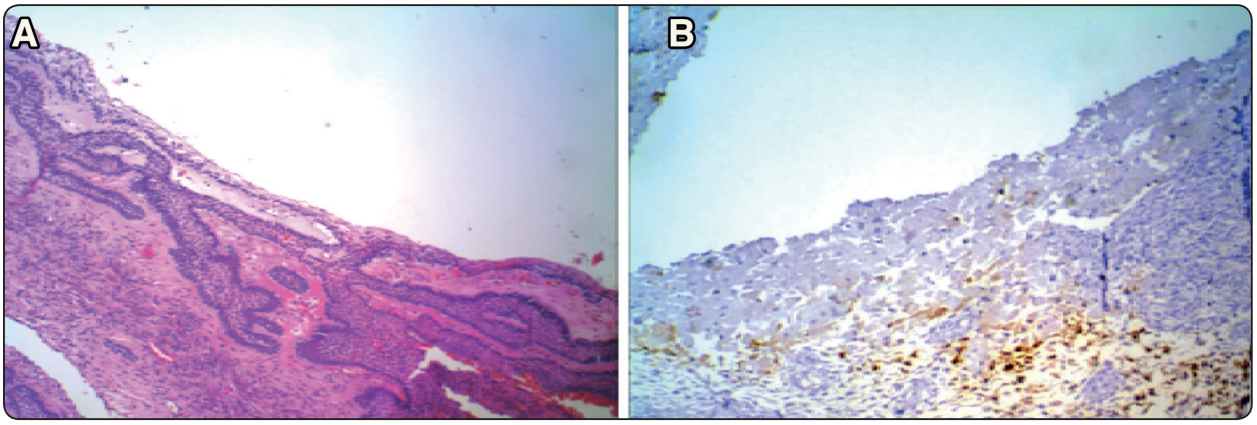

Fig. (2) H&E section showing unicystic ameloblastoma (A) (X100), Calretinin expression in unicystic ameloblastoma showing moderate staining reaction (B) (X200)

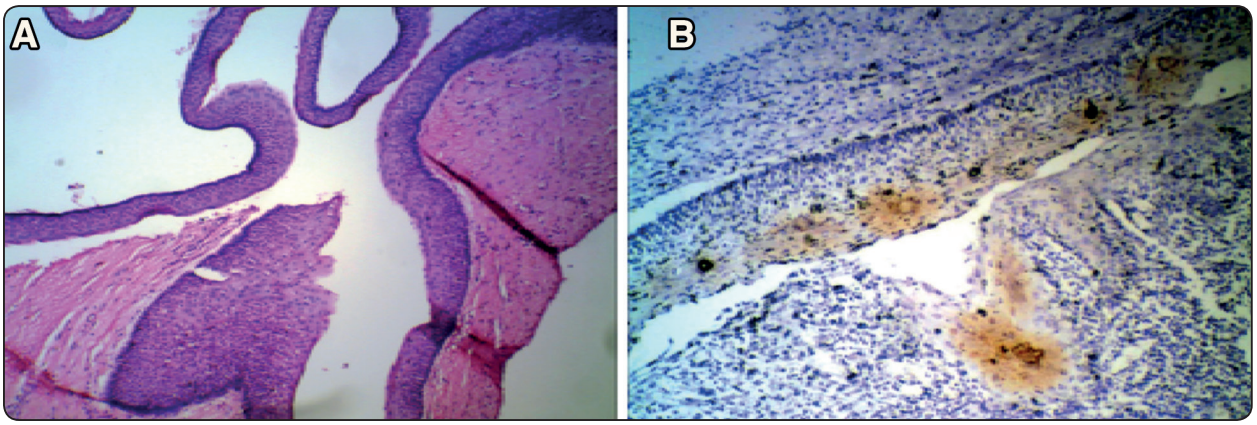

Fig. (3) H&E section showing Keratocystic odontogenic tumor (A) (X100), Calretinin expression in Keratocystic odontogenic tumor showing mild staining reaction (B) (X200)

TABLE (1) Calretinin Expression and Percentage of Positivity

Case	Total Number	Positive cases	Percentage
Periapical cyst	8	0	0
Dentigerous cyst	7	0	0
Keratocystic odontogenic tumor	7	4	57%
Solid ameloblastoma	8	8	100%
unicystic ameloblastoma adenomatoid	6	4	67%
odontogenic tumor	6	0	0

DISSCUSION

Odontogenic cysts and tumors have variable clinical and biological behaviours. 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. Unicystic ameloblastoma is a neoplasm with cyst like behaviour while keratocystic odontogenic tumor is an aggressive cyst with neoplastic behaviour. Keratocystic odontogenic tumor arises from cell rests of the dental lamina, same origin as ameloblastoma, and there are clinical and radiographic similarities between keratocystic odontogenic tumor and unicystic ameloblastoma, as both present as ordinary cysts.[7,15]

Histologically, unicystic ameloblastoma is lined in some areas by odontogenic epithelium of ameloblastoma appearance and stratified squamous epithelium in the remaining areas.[16] In fact, such squamous metaplasia is a relatively frequent phenomenon in unicystic ameloblastoma and many of these lesions are lined by such epithelium, which can create diagnostic confusion with other odontogenic cysts.[10] Rosenstein et al.[17] showed that cystic ameloblastoma could be difficult to identify microscopically and misdiagnosed as dentigerous cyst or other type of developmental cysts.

Keratocystic odontogenic tumor is characterized, histologically, by a palisaded basal cell layer of basophilic columnar cells and a surface of corrugated parakeratin, sometimes with spongiosis, resembling closely the stellate reticulum and the acanthomatous differentiation of ameloblastoma. If the tissue sample is small and if the neoplastic epithelium displays reactive changes induced by inflammation, it can closely resemble unicystic ameloblastoma. Thus, at times, both lesions become histologically indistinguishable.[18] Many techniques have been used in an attempt to distinguish odontogenic cysts (including KCOT) from ameloblastomas (especially unicystic type), while differences have been shown to occur between various cysts and ameloblastomas, considerable overlap exists and none of these techniques can be used to routinely distinguish these lesions from one another.[13]

Intracellular calcium ions are considered to be important second messengers intervening in several cellular processes, including proliferation and differentiation.[19] Calretinin is a calcium-binding protein of the EF-hand family and is expressed abundantly in central and peripheral neural tissues.[20] The exact biological function of calretinin remains unknown but its possible roles as a calcium buffer and/or calcium sensor and regulator of apoptosis have been postulated.[13]

It has recently emerged as an immunohistochemical marker with great utility for differential diagnosis in specific areas of pathology. Calretinin expression has also been investigated in many normal human tissues and other human neoplasms, but its potential role as a specific immunohistochemical marker of these tissues has yet to be fully elucidated. This protein is considered to be a definitive marker for mesothelioma (malignant tumor of the lining that covers internal organs) and has also been demonstrated in some carcinomas and adenocarcinomas of the lung, breast, pancreas and ovary.[21,22]

Calretinin has been demonstrated in the odontogenic epithelium during odontogenesis in tooth germs of rats suggesting that calretinin may play a role in their differentiation.[14] Coleman et al. [10] demonstrated that calretinin was found to be a specific immunohistochamical marker for ameloblastic epithelium and may have a role in the transition of the epithelial lining of odontogenic cyst to ameloblastomatous epithelium. Moreover, calretinin may serve as a diagnostic tool for differentiating cystic odontogenic lesions from ameloblastic tumors.

The present study was carried out mainly to evaluate calretinin expression in various odontogenic cysts and odontogenic tumors. The current study showed that calretinin expression was observed

in solid (multicystic) ameloblastoma, unicystic ameloblastoma and keratocystic odontogenic tumor. The immunopositivity was seen almost exclusively in the stellate reticulum of the studied cases of solid ameloblastoma while the peripheral layers of the ameloblastic islands were negative. In unicystic ameloblastoma, the reactivity was limited to the stellate reticulum like area. Calretinin expression was also observed in the lining epithelium of keratocystic odontogenic tumor. This was similar to the findings of Altini et al., [2] who indicated that calretinin expression in some cells varied according to their metabolic activity and may be lost when this activity changes and Coleman et al.[10] who showed that calretinin is expressed in the odontogenic epithelium of tooth germs at various stages of development. The authors attribute these findings to the aggressive biologic behaviour of these neoplasms and the high mitotic activity as compared to other non-neoplastic odontogenic cysts. [23] This means that calretinin may have a role in the transition of the dental lamina remnants to these lesions. It might also influence the difference in behaviour observed between this tumors and other odontogenic tumors.[11]

The other lesions in the present study showed no immunoreactivity for calretinin which was in accordance with the study conducted by Alaeddini et al.[11] and D'Silva et al.[23] It may be postulated that the role of calretinin in the development of these lesions is minimal and duration of calretinin expression in the process of histodifferentiation is limited and cannot therefore be detected by conventional immunohistochemical methods.

In conclusion, considering that ameloblastomas and keratocystic odontogenic tumor were consistently reactive for calretinin, whereas the other studied cysts and tumors were invariably non-reactive, these findings helps us to understand the importance of calretinin as a differential diagnostic marker for these tumors and how important it is for the pathologist to differentiate these lesions which carry different treatment protocols with potentially serious functional and esthetic consequences for the patient. So, it can be hypothesized that calretinin may have a role in the pathogenesis of these aggressive neoplasms and can be consider as an important diagnostic adjunct in the differential diagnosis between these neoplasms and other odontogenic tumors.

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