# CALRETININ EXPRESSION IN THE DIFFERENTIAL DIAGNOSIS OF AMELOBLASTOMA AND KERATOCYSTIC ODONTOGENIC TUMOUR

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## ABSTRACT

***Objective:*** To determine calretinin expression by immunohistochemistry in ameloblastoma and keratocystic odontogenic tumors (KCOT) and to document the use of calretinin as a differentiating marker between the two lesions.

***Study Design:*** A cross sectional study conducted on previously diagnosed cases of ameloblastoma and Keratocystic odontogenic tumour.

***Place and Duration of Study:*** Armed forces Institute of Pathology, Rawalpindi Pakistan and duration was one year. (Sep 2009- Aug 2010).

***Materials and Methods:*** Twenty cases each of Ameloblastoma and KCOT were retrieved from the record files along with their paraffin embedded blocks. Histological features of all the cases were reviewed on freshly prepared slides and a fresh diagnosis made regardless of the previous diagnosis. The immunohistochemical marker, Calretinin, was applied on both types of cases using the avidin-biotinylated peroxidase complex method. The results were interpreted.

***Results***: In the cases of Ameloblastoma the epithelial tumour nests showed positivity for Calretinin expression. In 85% cases; intense and diffuse staining was observed in more than 80% of the stellate reticulum like cells while 15% cases showed focal and moderate staining patterns. On the other hand KCOT showed contrary results as none of epithelial lining expressed positive staining for Calretinin, ($p$<0.001).

***Conclusion:*** Calretinin can be used as a useful marker for Ameloblastoma and can be used to differentiate KCOT from Ameloblastoma.

**Keywords:** Ameloblastoma, Calretinin, Immunohistochemical marker, Keratocystic odontogenic tumour.

## INTRODUCTION

Ameloblastoma is a borderline tumour as it is locally invasive and rarely metastasizes. It has higher recurrence rate than odontogenic cysts after surgical removal[1-3]. Keratocystic Odontogenic tumour (KCOT), previously called odontogenic keratocyst, has been renamed in the World Health Organization (WHO) 2005 histological classification of odontogenic tumours to highlight its neoplastic nature[3-5]. Though it has recurrence potential but it does not metastasize. In the presence of inflammatory changes KCOT can be histologically misdiagnosed as ameloblastoma. Sometimes ameloblastoma can be misinterpreted as KCOT because of insufficient biopsy sample. So both pathologic entities can be misdiagnosed for each other[5]. Whenever the morphological patterns mimic each other, it becomes difficult for the histopathologist to make a definitive diagnosis by morphology alone and Immunohistochemistry is a very helpful diagnostic tool.

There is a need for a diagnostic aid other than light microscopy to differentiate these two entities which have entirely different biological behavior and surgical protocol. Different immunochemical markers have proven their significance while others are being investigated.

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Calretinin, a 29-Kilodalton calcium binding protein belongs to large family of proteins which also includes S-100 proteins. Structurally it is characterized by 6 helix loop helix folds. These folds are binding sites of calcium[5-6]. The biological role of Calretinin is unknown while its possible role as calcium buffer and regulator of apoptosis has been proposed. Its role as most specific and sensitive marker of both benign and malignant mesotheliomas has been well established which is nearly 100%[7-9]. Expression of Calretinin is useful in central and peripheral neural tissues, convoluted tubules of kidney, Leydig and Sertoli cells of the testis, endometrium, ovarian stromal cells and adrenal cortical cells[9-12]. Contrarily, a small percentage of adenocarcinomas of different origins are weakly reactive to calretinin[13]. Calretinin expression was observed in the epithelium-derived tissues during process of odonotogenesis in rat molar tooth germs. It showed that preameloblast stained more intensely as compared to secretory ameloblasts[14]. Its role is also being investigated in diagnosing Ameloblastoma as its diagnosis becomes difficult if there is metastasis to other organs[15]. Studies are being conducted worldwide in order to establish the role of calretinin in diagnosing ameloblastoma and favorable results are being documented showing positive epithelial staining of calretinin in ameloblastoma. At times KCOT resembles ameloblastoma and poses a diagnostic challenge for the pathologist; therefore, use of calretinin is being studied in various parts of the world as an adjunct to differentiate the two pathologies. Thus, we selected this study to investigate the valuable potential of Calretinin in differentiating ameloblastoma and KCOT in our setup as there are no studies from this part so that we can compare the immunoreactivity of calretinin with the results of the other studies going on worldwide.

The purpose of this study was to establish whether calretinin in ameloblastoma and KCOT can serve as a marker that would be useful in the differential diagnosis of ameloblastoma and KCOT.

## MATERIALS AND METHODS

It was a descriptive, study conducted at Department of Histopathology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan from Sep 2009 to Aug 2010. Total number of 40 cases was included in the study comprising of 20 previously histologically diagnosed cases of ameloblastoma and 20 previously histologically diagnosed cases of keratocystic odontogenic tumour.

**Table: Calretinin expression in ameloblastoma and Kcot tumer.**

| Nomenclature | Grading | | |
|---|---|---|---|
| Ameloblastoma (n = 20) | 0(0%) | 3(15%) | 17(85%) |
| KCOT (n = 20) | 20(100%) | 0(0%) | 0(0%) |

***p* < 0.001**

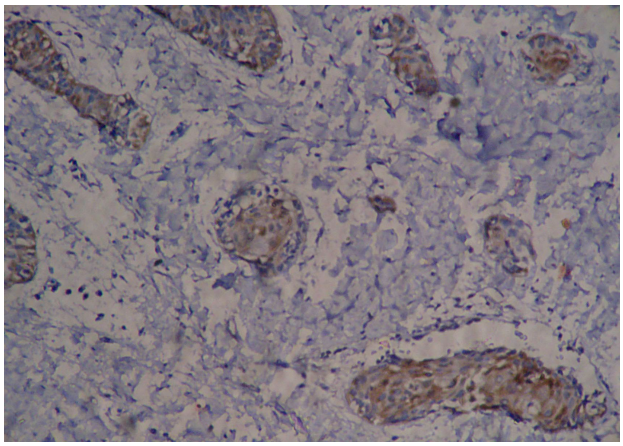

**Figure: Strong calretinin staining ameloblastoma*200.**

Non probability convenience sampling technique was used for sampling.

Blocks with scanty and autolysed tissues or poorly processed blocks were excluded. The data on gender, age and site of involvement and radiological findings were noted form clinical files in each case.

Histological features of all the cases were reviewed on freshly prepared slides form block samples and a fresh diagnosis was made

regardless of the previous diagnosis by using the conventional microscopy.

The immunohistochemical marker, calretinin was applied on both types of cases on 4 micrometer thick sections of selected cases of ameloblastoma and keratocystic odonotgenic tumour using polyclonal rabbit anticalretinin (Zymed Labs Inc. San Francisco, CA, USA) employing strepta-vidin-biotin complex immunoperoxidase technique. The slides were incubated with rabbit anti calretinin antibodies which is a purified form of rabbit anti sera and diluted in phosphate buffered saline with PH 7.4 and 1% bovine serum albumin with 0.1% sodium azide were used as preservative. Human brain tissue was used as positive control for calretinin. For negative control the primary antibody was replaced with normal serum. Immune reactivity was evaluated by assessing the percentage of positive cells along with pattern, localization in the cell and intensity of staining, using conventional light microscopy.

Age was taken as quantitative variable whereas site, sex, microscopic features of Ameloblastoma and keratocystic odonotgenic tumour and pattern of immune reactivity in term of percentage and intensity were our qualitative variables.

Data had been analyzed using SPSS version 15. Descriptive statistics were used to describe results. Chi- squre test was applied to compare calretinin expression between ameloblastoma and K & T cases. A $p$ value < 0.05 was considered as significant.

## RESULTS

Patients affected by Ameloblastoma were 60% males and 40% females with male to female ratio of 1.5:1. In case of KCOT, there were 55% males and 45% females with male to female ratio of 1.22:1.

The age range for ameloblastoma was between 10-77 years with mean of 31. The age range for KCOT was between 12-41 years with a mean of 25.

The most common site of tumour presentation in Ameloblastoma was mandible making upto 98% followed by maxillary ridge 2%. In KCOT the most common site of tumour presentation was also mandible.

Out of 20 cases of ameloblastoma, thirteen (65%) were of follicular and plexiform with equal frequency, two (10%) were of granular cell variant, two (10%) were arising in the dentigerous cyst, two (10%) were of acanthotic variant with squamous differentiation and one (5%) was of unicystic ameloblastoma.

The staining with Calretinin was diffuse in seventeen cases (Fig) and focal in three that was not dependent on the histological types, except for one case of unicystic ameloblastoma which showed focal pattern.

In two cases of ameloblastoma arising in dentigerous cyst the staining was positive in areas showing ameloblastic features and the epithelial lining of dentigerous cyst showed negative staining. The Calretinin expression was intense in the epithelium lining the micro or macrocysts. The intense staining of the macrocyst occur even in the absence of epithelial lining.

In all 20 cases of KCOT, the epithelial lining of the cyst showed no positive staining in all (100%) cases. Calretinin expression was significantly different in ameloblastoma and KCOT cases ($p$<0.001).

## DISCUSSION

The differential diagnosis of primary KCOT and ameloblastoma is challenging especially in small incisional biopsies as clinical, radiological and histopathological presentation could be equivocal. An attempt is made in this study to establish the role of calretinin in the differential diagnosis.

Distribution of KCOT was found higher in males in our study. Similar findings were observed in studies by Gupta et al and Pitak etal (2010)[16-17]. Mean age distribution was almost similar to the studies from California and Northern China 29.4 and 28.56 respectively.

Higher mean age was found by Gupta and poninnah[16]. Like other studies mandible was found to be the most frequent site of involvement by KCOT[16].

Neville (2002) reported equal precipitation for both males and female in ameloblastoma[3]. Our study found increased frequency in males. Wide age range has also been reported by Neville similar to the patients observed in our study that ranged between 10-77 years. Like other studies mandible was found to be the most frequent site of involvement of ameloblastoma[18].

Radigraphically both KCOT and Ameloblastoma appear as unilocular or multilocular cysts, which is the picture in most reported cases.

Regarding calretinin expression the results of our study have demonstrated intense positive expression of Calretinin in stellate reticulum present in central core of tumour island. Whereas all the cases of KCOT showed negative expression for calretinin.

The results of our study showed similar results as that of Altini at al (2000) who showed 29 of the 31 ameloblastoma to be positive for calretinin with intense staining stellate reticulum–like cells[19]. De Villiers et al (2008) also observed 100% positive staining in 19 cases of ameloblastoma while 17 cases of KCOT were 100% negative for the stain. They observed the staining pattern restricted to the neoplastic epithelial counterparts[5].

Piattelli et al (2003) investigated Calretinin expression in odontogenic cysts in U.S.A including twenty four radicular cysts, twenty four follicular cysts and twenty two Odontogenic keratocystic tumours. All the radicular cysts, follicular cysts and all Odontogenic keratocystic tumours showed negative expression for calretinin both in epithelial and stromal components[18].

Coleman et al (2001) observed lining epithelium of eight cases of unicystic ameloblastoma, six cases of dentigerous cyst, six cases of KCOT, and four cases of solid/multicystic ameloblastoma for the expression of calretinin. No positive staining was observed in any of the dentigerous cysts and keratocystic odontogenic tumor linings. In comparison, coarse dark brown staining was seen in the stellate reticulum of solid multicystic ameloblastoma and more superficial epithelial layers of unicystic ameloblastoma. Thus they established calretinin as a specific immunohistochemical marker for neoplastic ameloblastic tissue that can be used as an important diagnostic aid in the differential diagnosis of unicystic ameloblastoma and cystic odontogenic lesions[20].

In Iran Alaeddini et al (2008) studied comparative expression of Calretinin in selected odontogenic tumors. Total 55 odontogenic tumours were assessed for calretinin expression. They include 20 cases of Ameloblastoma, 5 calcifying epithelial odontogenic tumour, 10 adenomatoid odontogenic tumours, 10 ameloblastic fibromas and 10 cases of odontogenic myxomas. All cases of ameloblastomas showed positive staining for calretinin while remaining tumours were negative for the stain. The difference in proportion for the calretinin expression in the two groups was significant[21].

## CONCLUSION

The ameloblastomas, in contrast to KCOT were consistently reactive for Calretinin, therefore, it may be an important and useful diagnostic aid to differentiate ameloblastoma from KCOT.

### Conflict of Interest

This study has no conflict of interest to declare by any author.

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