

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN  
CHEMISTRY AND PHARMACEUTICAL SCIENCES**

(p-ISSN: 2348-5213; e-ISSN: 2348-5221)

[www.ijcrpps.com](http://www.ijcrpps.com)

DOI:10.22192/ijcrpps

Coden: IJCROO(USA)

Volume 3, Issue 10 - 2016

**Research Article**



DOI: <http://dx.doi.org/10.22192/ijcrpps.2016.03.10.001>

**Immunohistochemical Comparison of CD10 Expression  
in ameloblastoma, odontogenic keratocyst and  
radicular cyst**

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**Abstract**

**Objective:** The goal of this study is to compare expression of CD10 in the epithelial part and stromal tissue of odontogenic keratocysts, radicular cysts and ameloblastomas.

**Material and Method:** in this observational and cross-sectional study, immunohistochemical staining of CD10 on formalin-fixed, paraffin-embedded tissue sections of odontogenic keratocysts (n=11), radicular cysts (n=11) and ameloblastoma (n=11) was performed by standard Envision method. Then, slides were studied to evaluate the following parameters: expression in epithelial part, stromal tissue, gender, mean age and location of the lesion.

**Results:** The data analysis showed statistically significant difference in CD10 expression in stromal tissue among studied groups ( $p \leq 0/01$ ), with more numbers of cases in Ameloblastoma and OKC respectively.

**Conclusion:** CD10 expression was more in ameloblastoma and OKC compared to radicular cysts. These results suggest that high expression of CD10 in ameloblastoma and OKC might be a reason for aggressiveness of AB and recurrence of OKC. It also proposes the neoplastic origin of odontogenic keratocysts.

**Keywords:** CD10, Odontogenic keratocyst, Radicular cyst, Ameloblastoma, Immunohistochemistry

**Introduction**

Different types of odontogenic cysts and tumors originate from remnants of dental lamina. The capacity for additional proliferation of these epithelial remnants during cyst formation is different and thus cause variations in their biological behavior and molecular expression, due to an unknown mechanism (1). Ameloblastoma is the most common odontogenic tumor(2). This tumor arises from epithelial cell rest of Malassez after regression of the enamel organ (3). OKCs are known for their extensive local invasion into

adjacent structure and high recurrence rate after treatment(5,6). These clinical features are also seen in ameloblastoma, which show involvement of adjacent soft tissue and destructive growths. In addition to locally invasive behavior, both characterized by a high rate of recurrence. For these reasons, OKC has been named "keratocystic odontogenic tumour" by the World Health Organization (WHO) classification of Head and Neck Tumors in 2005(7). However, there is still no consensus about whether OKC is a benign neoplasm or

not. Radicular cyst are the most common inflammatory cyst of the jaws and arises from proliferation of small odontogenic epithelial residues. They do not recur after appropriate management(5,6).

CD10 is an endopeptidase with zinc-dependent metalloproteinase enzymatic activity. Stromal CD10 expression is associated with tumour cell differentiation and proliferation. Some studies have demonstrated a significant correlation between CD10 expression and tumour progression and metastatic dissemination for certain type of cancer, breast cancer, colorectal cancer and malignant melanoma.(8)

In the present study, expression of CD10 in odontogenic keratocyst, radicular cyst and ameloblastoma in order to evaluate and compare proliferative activity in these lesions was undertaken.

## Materials and Methods

### Sample collection

After reviewing clinical information and histologic findings, a total of 33 odontogenic cysts and tumors consisting OKCs (n=11), RCs (n=11) and ameloblastoma (n=11) were collected from paraffin-embedded blocks of Oral and Maxillofacial Pathology Department, Jundishapur University of Medical Sciences, Ahvaz, Iran.

The inclusion criteria were OKC & ameloblastoma without excessive tissue hemorrhage and only for OKC samples consisted of the presence of the epithelium of the cyst wall, with minimum inflammation in the cyst wall. Inflammatory lesions except for radicular cysts because of probable effect of inflammation on proliferative activity were excluded from the study.

### Immunohistochemistry

IHC staining of paraffin blocks was as follow:

IHC staining was performed by standard Envision methods. After taking slices, samples were placed on slides stained with Poly-L-Lysin deployed for 24 hours at 37°C to dry. The samples were then deparaffinized in Xylene and rehydrated in varying degrees of ethanol. Consequently, in order to stop the inner peroxidase activity, samples were placed in methanol containing peroxide (H<sub>2</sub>O<sub>2</sub>) 0.3% for 30 minutes at room temperature and then rinsed in Phosphate buffered saline (PBS) solution PH = 7.2. Immunohistochemical staining for cyclin D1 (Biocare / USA / monoclonal/ Clones:56c6/ Dilutions: 1:50-1:100) were performed according to the manufacturer's recommendations. After the incubation with the primary antibody, Envision technique was used. Samples were incubated with

Polymer solution (anti-mouse) for 30 minutes and washed with PBS. In the next stage, the

3,3 DiaminobenzidineHydrochloride (DAB) dye that gives brown color to antigen-antibody complex was used. Samples were counterstained with hematoxylin and plates were placed on them. Finally, the immunohistochemical staining status was analyzed by optical microscope by two pathologists. In this study, Tissue sections of the oral squamous cell carcinoma were considered as positive control and an odontogenic lesion without adding the primary antibody was used as a negative control.

### Staining evaluation:

All slides were reviewed by two pathologists blindly. Evaluation of samples was performed by assessing the intensity and percentage of stained cells. For each positive section, 5 microscopic fields which showed highest immunoreactivity in epithelial and stromal cells were identified by x40 magnification and the number of positive cells was divided into the total number of cells counted in every field. Then the average of all fields was calculated. The result was multiplied by 100 to find the percentage of positive cells. Because in our samples, RC did not have adequate lining tissue, we have used this method to calculate positive cells. Data were scored according to Abdel-aziz's study (8) as follow:

1. Negative: Brown membranous and cytoplasmic staining of stromal cells <10%
2. Positive: Brown membranous and cytoplasmic staining of stromal cells more than 10%
1. Low: Brown membranous and cytoplasmic staining of stromal cells 10-25%
2. Intermediate: Brown membranous and cytoplasmic staining of stromal cells 25-50%
3. High: Brown membranous and cytoplasmic staining of stromal cells more than 50%

### Statistical analysis

Analysis of the data was performed using Statistical Package for Social Sciences (SPSS software) (SPSS, Inc, Chicago, IL, USA) version 18.0. The Chi-Squared test was used for all variables. Mann-Whitney test was performed for pairwise comparison of expressed cells in stromal tissue and T-test for that in epithelial part among groups. P<0.05 was regarded as statistically significant.

## Results

Results are as follows:

As Table II shows, CD10 expressed in epithelial part of 11 (63.6%) cases and stromal tissue of all cases with more cases of intermediate intensity in both groups.

In amelobelastomas, CD10 was detected in epithelial part of 9 (45.5%) cases and stromal tissue of all cases with more cases (45.5%) of intermediate intensity in epithelial part and high intermediate intensity in stromal tissue of cases (54.5%).

CD10 was discernible in epithelial part of 10 (63.6%) cases and stromal tissue of all cases. As table II shows 7 cases (63.6%) had intermediate intensity in epithelial part and 6 cases (54.5%) of low intensity in stromal tissue.

Most of the cases were located in mandible (n=19). Mean age in OKCs, RCs and AB was 41, 36 and 29 years respectively. Lesions were more common in male (n=22). (Table I)

Based on Table III, the data analysis showed statistically significant difference in CD10 expression in stromal tissue of studied groups (p=0.001), (p<0.05, Chi-Squared test). Moreover, pairwise comparison showed, the difference in CD10 expression between OKCs and RCs (p=0.01), as well as Amelobelastomas and RCs (p=0.047), but the statistically difference was not observed between OKCs and Amelobelastomas (p=0.478), (p<0.05, Mann-whitney test).

As Table III shows, there is no statistical difference in epithelial expression among studied groups. Amelobelastomas had the most cases of high expressed intensity in epithelial part (36.4%) among three groups. RCs and OKCs showed equally intermediate intensity (63.6%), but based on Chi-squared test, there was no statistically significant difference (p=0.530).

**Table I: demographic information of studied groups**

	Mean age	Gender		Site	
		Male	Female	Maxilla	Mandible
<b>OKC</b>	41	9(81.8%)	2(18.2%)	5(45.5%)	6(54.5%)
<b>RC</b>	36.54	7(63.6%)	4(36.4%)	7(63.6%)	4(36.4%)
<b>AB</b>	29.63	6(54.4%)	5(45.5%)	2(18.2%)	9(81.8%)

(OKC) odontogenic keratocyst, (RC) radicular cyst, (AB) amelobelastoma

**Table II: Distribution of epithelial and stromal expression, location of lesion, gender and mean age of all groups of lesions**

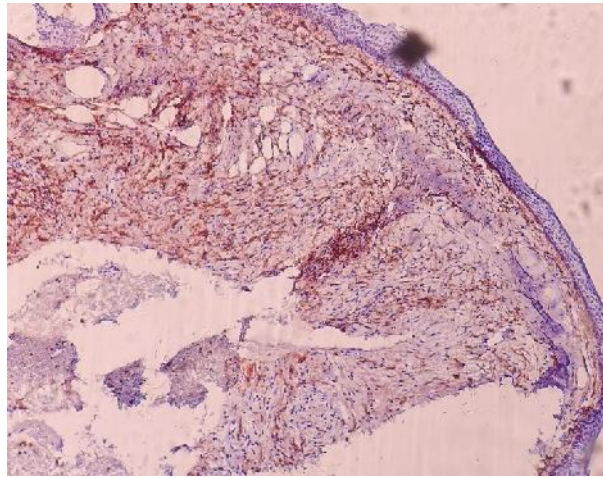
	<b>OKC</b>	<b>RCs</b>	<b>AB</b>	<b>P value</b>
	n (%)	n (%)	n (%)	
<b>Epithelial expression</b>				0.530
No staining	3(27.3)	1(9.1)	2(18.2)	
Low	0(0)	0(0)	0(0)	
Intermediate	7(63.7)	7(63.7)	5(45.5)	
High	1(9.1)	3(27.3)	4(36.4)	
<b>Stromal expression</b>				0.001
No staining	0(0)	0(0)	0(0)	
Low	0(0)	6(54.5)	4(36.4)	
Intermediate	9(81.8)	5(45.5)	1(9.1)	
High	2(18.2)	0(0)	6(54.5)	

(OKC) odontogenic keratocyst, (RC) radicular cyst, (AB) amelobelastoma

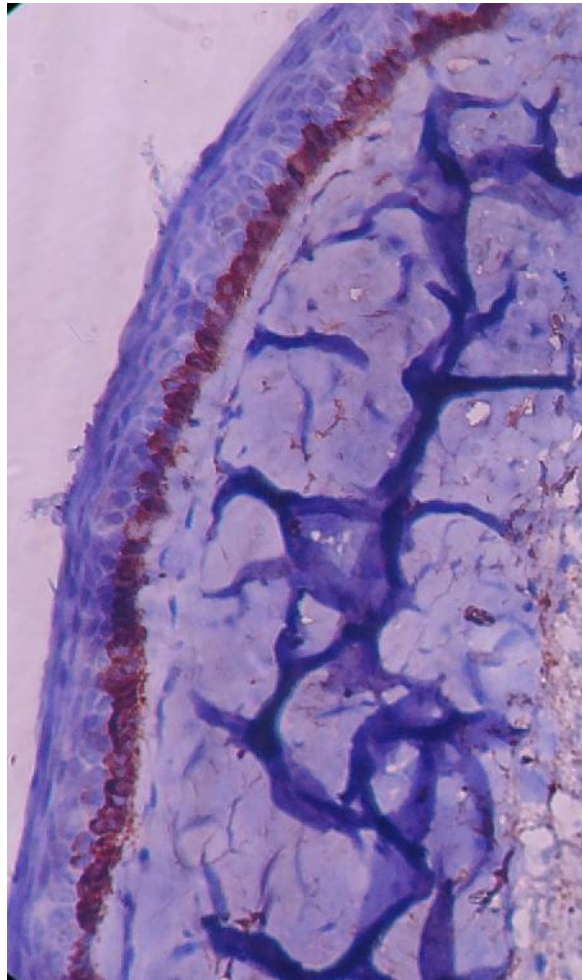
**Table III: Average of expressed cells (%)**

Lesion	Stromal expression		P value	
	OKC (35.09)	AB (40.90)		
	OKC (35.09)	RC (14.81)	0.010	
	AB (40.90)	RC (14.81)	0.047	
Lesion	Epithelial expression		P value	
	OKC (32.80)	AB (37.63)		0.678
	OKC (32.80)	RC (30.90)		0.767
	AB (37.63)	RC (30.90)		0.470

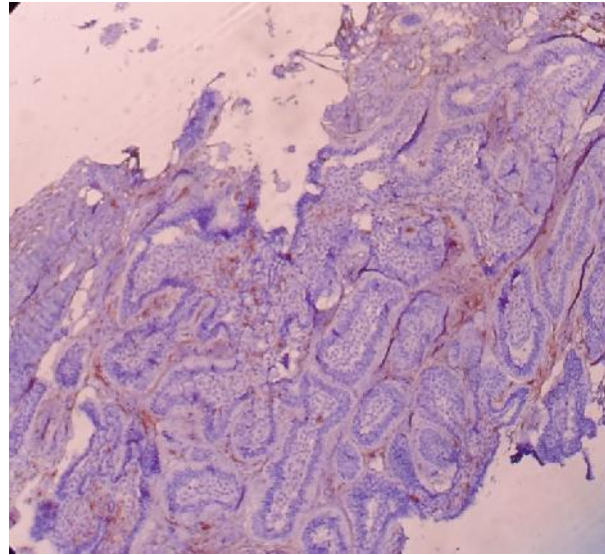
(RC) radicular cyst, (OKC) odontogenic keratocyst, (AB) amelobelastoma



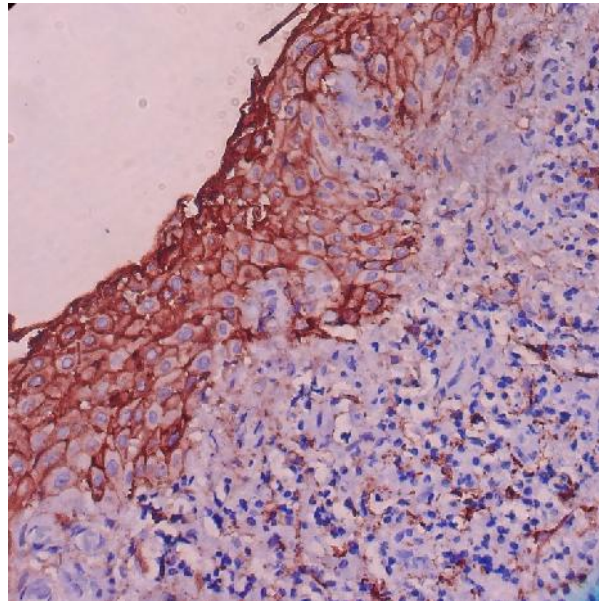
**Figure 1.** CD10 immunoreactivity in epithelial and stromal cells of OKC (x10)



**Figure 2.** CD10 immunoreactivity in epithelial cells of OKC (x40)



**Figure 3.** CD10 immunoreactivity in stromal cells of ameloblastoma (x10)



**Figure 4.** CD10 immunoreactivity in epithelial and stromal cells of RC (x40)

## Discussion

The classification of odontogenic jaw cysts is based on their histology, pathogenesis and presumed developmental origin. Radicular cysts have an epithelial part derived from the epithelial rests of Malassez. It is presumed that epithelia of OKCs originates from the odontogenic epithelium of the dental lamina or its remnants, prior to tooth formation. However, it has also been suggested that odontogenickeratocysts could be derived from basal cells of the oral mucosa (9). Odontogenickeratocysts show a particular tendency to recur after their surgical

removal that could be due to a multilocular nature or a presence of daughter or satellite cysts that are left behind. However, it has also been hypothesized that the epithelium of these cysts could have an intrinsic potential to growth, resembling benign neoplasms (10). Ameloblastomas are tumors that arise from rests of the dental lamina. Thus, OKCs and ameloblastomas derived from the same cell population, and like ameloblastomas, OKCs may behave aggressively and can penetrate cortical bone, extending into the surrounding tissues, with also they show recurrence rates ranging from 3% to 60% (11).

CD10 is 90-110 kDa cell surface zinc dependent metalloprotease which possesses a well-defined enzymatic activity where it cleaves and inactivates neuropeptides and peptide hormones at the amino terminus to hydrophobic residues within the peptide sequences, thereby decreasing the cellular response to local peptide hormones. It has also been called as neutral endopeptidase, enkephalinase, neprilysin and common acute lymphoblastic leukemia antigen (CALLA) (12). CD10 is expressed on some normal and neoplastic haemopoietic, lymphoid and epithelial cells (13). CD10 expression has also been detected in tumor associated stromal cells indicating its vital role in tumor-stromal interactions (14).

In the present study, there was statistically significant difference in stromal expression across studied groups. But it did not differ between AB and OKC. That could probably be related with the biological aggressiveness of AB and OKCs. This is in line with Andishe tadbir A et al. (15). A study by Kanitakis *et al.* (20) showed an increase in the expression of CD10 during metastasis. They concluded that the expression of CD10 might be used for the differential diagnosis of primary and metastatic melanoma.

According to Table II, in this study stromal expression of CD10 in AB, OKC and RC is high, intermediate and low in most of the cases respectively. This increase in CD10 immunoreactivity might explain the variable behavior of these lesions and high aggressive and invasive behavior of ameloblastoma and OKCs and support the notion that OKC have a neoplastic nature. This result is similar to the results of Abdel-Aziz and Amin (8) and Iezzi *et al.* (12) who demonstrated that higher recurrence rate is seen in tumors with high stromal CD10 expression. In addition, high expression of CD10 was seen in samples metastasizing to axillary lymph nodes. A study by Ogawa *et al.* (17) did not show the expression of CD10 in the stromal cells of colorectal tissue; however, expression of CD10 in the stromal cells increased with an increase in dysplasia in the tumoral cells of adenoma. Stromal expression of CD10 exhibited a significant relationship with p53 aggregation and tumor size, consistent with the results of studies by Langner *et al.* (18) and Braham *et al.*, (19) carried out on renal and nasopharyngeal carcinomas, respectively. These studies showed that tumors with higher grades exhibited more severe staining of CD10 in the stromal cell, consistent with the results of the present study.

We observed no significant difference in epithelial expression, but it increased from RC to OKC and AB. This is in contrast with Andishe tadbir *et al.* (15) which found significant difference between studied groups except solid ameloblastoma and OKC. This difference may be due to retrospective sample collecting. This means that the blocks between the

years 1388-1394 were used. Perhaps the type of fixation and time of it affected divisiveness.

## Conclusion

Based upon the results of the present study, it could be concluded that, the absence of any significant differences in the expression of CD10 between AB and OKC might indicate the neoplastic potential and the tumor-like behavior of OKC. We also observed in this study that connective tissue cells were important cells in the biological behavior of these lesions. It is suggested that, this observation should be further investigated in larger series.

## Acknowledgments

This study was supported by Ahvaz Jundishapur University of Medical Science.

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**How to cite this article:**

Babak Karimi, Homadokht Banaie, Mahsa Saidian. (2016). Immunohistochemical Comparison of CD10 Expression in ameloblastoma, odontogenic keratocyst and radicular cyst. Int. J. Curr. Res. Chem. Pharm. Sci. 3(10): 1-7.

DOI: <http://dx.doi.org/10.22192/ijcrops.2016.03.10.001>