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Original Article

Comparison of immunohistochemical expression of CD10 in keratocystic odontogenic tumor and ameloblastoma

Elham Hormozi[1], Vahid Nourollahi Fard[1], Mohammad Ali Naseri[1], Nima Haghighat Jahromi[1], Forooz Keshani[2]

[1]Department of Orthodontics, Faculty of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, [2]Torabinejad Dental Research Center and Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Background: Odontogenic keratocyst (OKC), also called keratocystic odontogenic tumor (KCOT), is a developmental lesion which should be carefully monitored and it exhibits development mechanisms and biologic behaviors different from those of other more common lesions such as dentigerous and radicular cysts. CD10 antigen is a cell surface metalloendopeptidase, which inactivates various peptides that are physiologically active. Studies have shown that increase in the expression of CD10 in the stromal cells helps the progression of the tumor. Ameloblastoma (AB) is a local invasive tumor and given the role of supporting connective tissue stroma in the aggression and progression. The aim of the present study was to comparatively evaluate the expression of CD10 in the connective tissue stroma of AB and OKC as a KCOT.

Materials and Methods: In this retrospective, cross-sectional study, 14 paraffin blocks of KCOT and 9 of AB (7 multicystic and 2 unicystic) were evaluated with CD10 immunohistochemical expression in the connective tissue stroma of AB and the connective tissue wall of KCOT. The data were analyzed with Fisher's exact test ($P < 0.05$).

Results: In 8 samples of 9 AB and in 13 samples of 14 KOT lesions, expression of CD10 was shown. Fisher's exact test did not reveal any significant differences between these two lesions in the expression of CD10 ($P = 0.64$).

Conclusion: The results of this study propose that high expression rate of CD10 might be one of the reasons for the aggressive behavior of AB and high recurrence rate of OKC and reinforce the classification of OKC as an odontogenic tumor.

Key Words: Ameloblastoma, CD10, keratocystic, odontogenic tumor, odontogenic keratocyst

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Address for correspondence:
Dr. Forooz Keshani,
Department of Oral and Maxillofacial Pathology, Torabinejad Dental Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: kforuz@yahoo.com

INTRODUCTION

Odontogenic keratocyst (OKC) is a developmental cyst with histopathological characteristics and local invasive behavior that necessitates special attention. The biologic behavior of OKC is different from those of other more common cysts such as dentigerous and radicular cysts.[1,2] OKC has been referred to as OKC tumor in the latest classification of odontogenic tumors by the WHO, which might be explained by generic molecular neoplastic changes of this cyst. Contrary to other cysts, OKC has a high recurrence potential, with a recurrence rate of approximately 30%.[3] Ameloblastoma (AB) is the most prevalent

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tumor with clinical significance because its relative frequency is equal to the sum of the frequencies of other odontogenic tumors, excluding odontoma. In addition, AB is a resistant infiltrative neoplasm which might lead to mortality by progressive expansion to involve vital structures. The tumor exhibits a poor prognosis in the posterior maxilla, with recurrence rates of 50-90% and 15% after curettage and resection, respectively.[3]

Metalloproteinases are a member of the metallopeptidase family, which degrade the protein complexes of the extracellular matrix and have a principal role in tissue regeneration.[4] Little attention has been paid to the role of proteases in the stem cell biology compared to the great role of extracellular matrix enzymes in the tumor metastasis.[1]

CD10 is a zinc-dependent metalloproteinase on the cell surface with a molecular weight of 90-110 kDa.[5] CD10 was first identified as a tumor-related antigen in human acute lymphocytic leukemia and other lymphoid malignancies with immature forms.[6] In fact, CD10 has been recognized as a membrane metalloendopeptidase, enkephalinase, neutral endopeptidase, neprilysin, and acute lymphocytic leukemia antigen, which distributes signaling peptide.[1] In addition, it leads to the local aggregation of some specific peptides.[6] Although the expression of CD10 has been observed in neoplastic cells, there are some reports about its expression in stromal cells. In addition, there are data indicating that expression of CD10 by stromal cells is associated with carcinogenesis and might be a new prognostic factor in some malignancies.[7]

The matrix has a key role in the development, invasion, and metastasis of the tumor through specific signals that lead to the proliferation, undifferentiation, and longevity of the tumoral cells.[4] Furthermore, neoplastic expression of CD10 has been reported in relation to the progression of the tumor and a decrease in patient survival in some malignancies such as biliary carcinoma, transitional cell carcinoma of the upper urinary tract, and endocrine tumors of the pancreas.[8] CD10 is normally expressed in different cells and tissues, including granulocytes, lymphoid germ cells, anthrocytes, fetal trophoblasts, glandular epithelium of the prostate gland, the epithelium of the gallbladder, myoepithelial cells, Schwann cells, and the epithelium of the renal tubules. In addition, it is expressed in the stromal cells of normal bone marrow and endometrium.[9] Therefore, CD10 might have an important role in the homeostasis, neoplastic changes, and progression of tumors. Based on recent research, expression of CD10 in tumor cells has apoptotic and proliferative roles.[6,9,10]

In 2012, Abdel-Aziz and Amin reported that expression CD10 and Ki-67 are significantly associated with recurrence and might help evaluate the biologic behavior of the tumor.[7]

In 2010, Oba *et al*. reported an increase in the expression of CD10 in malignant melanoma. They concluded that an increase in the expression of CD10 might be used as a marker for tumor progression.[8] Based on the results of previous studies, an increase in the expression of CD10 in the tumor stromal cells assists in tumor progression and is associated with poor prognosis in various tumors, including breast carcinoma, malignant melanoma, basal cell carcinoma, skin squamous cell carcinoma (SCC), and oral squamous cell carcinoma.[6,10]

Some researchers have suggested that OKC should be considered a benign cystic neoplasm rather than a cyst.

Andisheh-Tadbir and Fakharian evaluated heat shock proteins (HSP) 70 expression in dentigerous cyst, OKC, and AB and concluded that the expression rate of HSP 70 has a role in the pathogenesis of AB and OKC (keratocystic odontogenic tumor [KCOT]) and is one of the reasons for the aggressive behavior of AB and high recurrence role of OKC, reinforcing the classification of OKC (KCOT) as an odontogenic tumor.[11]

Deyhimi and Hashemzade had shown higher expression of p53 protein and transforming growth factor-alpha in OKC (KCOT) compared to those in orthokeratinized odontogenic cyst.[12]

Razavi *et al*. in their article had concluded that biological activities, high tendency to recur, and growth mechanisms of KCOT are different in comparison with other cystic lesions, which are related to apoptotic proteins. However, the aggressive potential of KCOT is not as severe as that of the neoplasms such as ameloblastoma.[13]

Since AB is a prevalent and aggressive tumor and OKC is a prevalent odontogenic tumor with an invasive behavior[5] and given the role of supporting connective tissue stroma in the progression of

tumors,[6] and also no study had shown expression of CD10 in KCOT; this study was undertaken to evaluate the expression of CD10 in the connective tissue stroma of AB and OKC.

MATERIALS AND METHODS

The present retrospective, analytical, and cross-sectional study were carried out in Jondishapur Faculty of Dentistry. Ahwaz, Iran in 2012. In the first stage, the OKC and AB lesions were selected by using the information registered in the files of patients had been referring to the Department of Oral and Maxillofacial Pathology of the Faculty and Jahad Pathology Center of Ahwaz. The samples consisted of incisional and excisional biopsies of the lesions in patient jaws, which had been fixed in 10% formalin, followed by the preparation of paraffin blocks. The H and E microscopic plates prepared from these lesions were reexamined for the evaluation of the quality and quantity of the tissue. 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. No specific ethical considerations were observed in the study due to the use of archived paraffin blocks, the absence of communication with the patients and no use of patients' names and particulars during the study procedures. After selecting suitable samples and recording the demographic and clinical data of the subjects, the samples underwent the standard envision immunohistochemistry (IHC) staining for CD10 marker using the DAKO kit (Denmark). After preparation sections, the samples were placed on slides coated with poly-l-lysine and left to dry at 37°C for 24 h. Then the samples were deparaffinized in xylene and rehydrated in various concentrations of ethanol. In order to inhibit the activity of intrinsic peroxidase, the samples were placed in methanol containing 0.3% peroxide for 30 min at room temperature and then rinsed with phosphate-buffered saline solution. In the next stage, the coloring agent, 3,3'-diaminobenzidine hydrochloride, which stains the Ag-Ab complex in brown, was used. The samples were then counterstained with hematoxylin, dehydrated, and covered. The lymphoid germinal center tissue was considered the positive control and the samples without antibody were considered the negative control.

Finally, the samples underwent histopathological evaluation and IHC staining for CD10 under a light microscope (CX21 Olympus, Japan) [Figures 1 and 2].

The expression of CD10 marker in the connective tissue stroma of ameloblastic lesions and the connective tissue wall of OKCs was evaluated. The following classification was used after evaluation of the slides:

- Negative: Brown membranous and cytoplasmic staining of stromal cells <10%
- Positive: Brown membranous and cytoplasmic staining of stromal cells more than 10%.[14]

For each positive section, ten microscopic fields which showed highest immunoreactivity in epithelial and stromal cells were identified by ×400 magnification and the percentage of stained cells was calculated.[15]

In addition, the severity of staining in the positive samples was classified as low, moderate, and high.[7] After evaluation of findings and calculation of descriptive values, the proportion of samples with low, moderate, and high CD10 expression were determined. Then Fisher's exact test was used to compare expression of CD10 between OKC (KCOT) and AB.

RESULTS

In the present IHC study, 9 AB (two unicystic, unicystic ameloblastoma (UA), and 7 multicystic

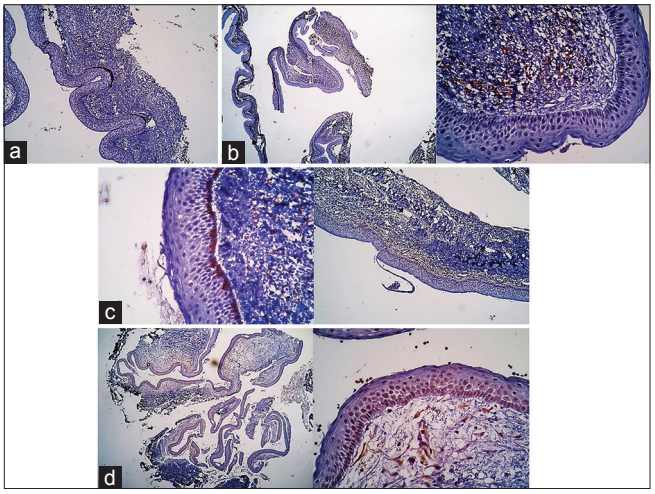


Figure 1: Represents cytoplasmic and membranous CD10 expression staining of odontogenic keratocyst for CD10: Pithelial cells did not stain for CD10; (b) staining for CD10 adjacent to the epithelium was moderate; (c) staining for CD10 adjacent to the epithelium was low and the basal membrane of the epithelium exhibited nucleus area staining; (d) the basal and parabasal cells of the epithelium exhibited staining for CD10 in the nucleus area.

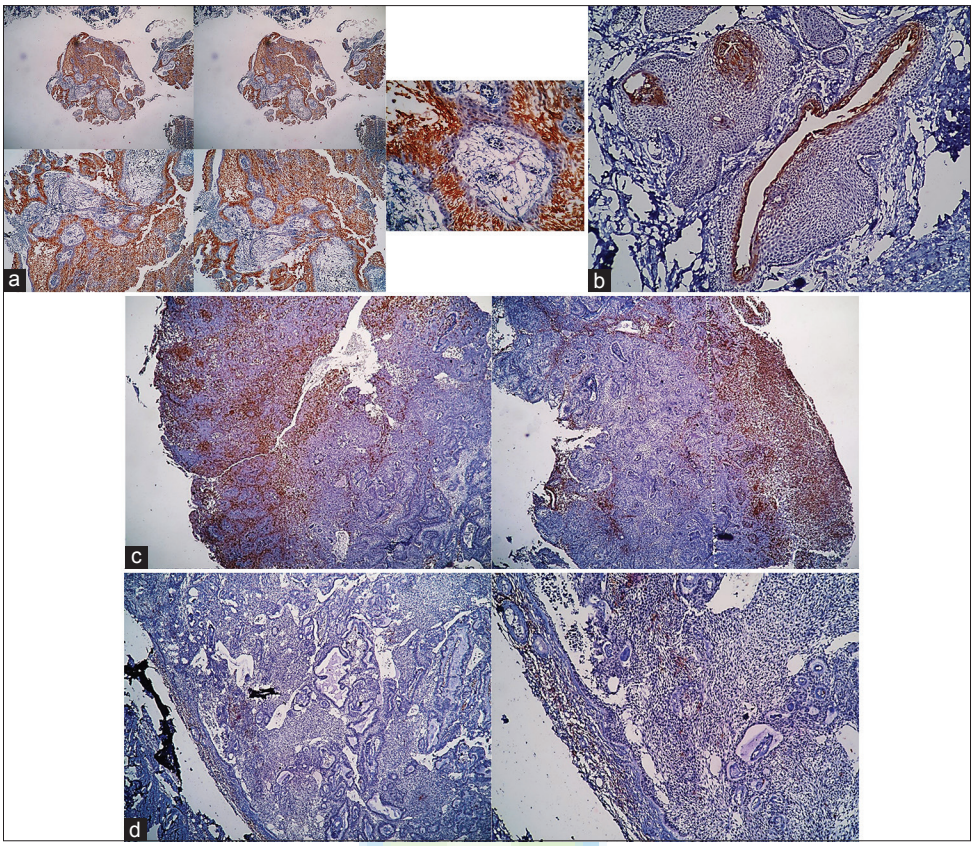


Figure 2: Represents cytoplasmic and membranous CD10 expression of ameloblastoma: (a) staining for CD10 adjacent to epithelial islands was high. Epithelial cells did not stain; (b) the epithelial cells within the stellate reticulum stained in the cytoplasm area; (c) staining was high between the islands and adjacent to them; (d) staining for CD10 was moderate in the connective tissue.

ameloblastoma [MCA], MCA, cases) and 14 KCOT lesions were evaluated in relation to the expression of CD10 marker. Demographic characteristic of lesions is showed in Table 1. CD10 expression was detected in 8 of 9 AB and in 13 of 14 OKC (KCOT) lesions, therefore it was negative in 1 KCOT and 1 AB samples [Graph 1].

Staining for CD10 was low, moderate, and high in stromal cells in 1, 4, and 3 AB samples, respectively. In OKC, such staining was low in 7, moderate in 5, and high in 1 of the stromal cells samples, respectively [Table 2]. From 2 UA samples, for CD 10 expression, one sample was negative and in other in the stromal cells showed low positively and also epithelial cells were nonstaining in both.No significant differences were detected in CD10 expression between OKC (KCOT) and AB samples (P = 0.64). P- and α-values were used to accept or reject the hypothesis of independence of the two variables.

Fisher's exact test was used to evaluate and compare expression of CD10 between AB and OKC (KCOT) lesions [Table 3].

Table 1: Demographic characteristic of lesions

Lesion	Gender		Age	
	Male	Female	Range	Mean
KCOT	4	10	18-50	33.64
AB	4	5	11-60	35

KCOT: Keratocystic odontogenic tumor; AB: Ameloblastoma

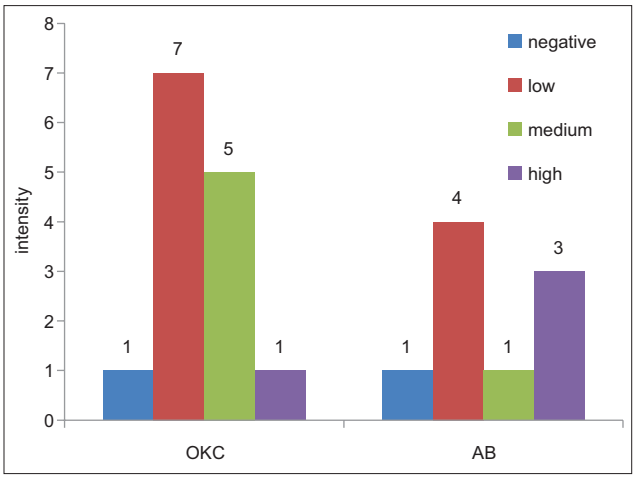
Table 2: Stromal expression of CD10 in different types of lesions

Type of lesion	Stromal expression			
	Negative	Low	Medium	High
AB	1	4	1	3
OKC	1	7	5	1

OKC: Odontogenic keratocyst; AB: Ameloblastoma

DISCUSSION

Odontogenic cysts and tumors comprise a large proportion of pathologic lesions in maxillofacial region, with a common origin from the odontogenic epithelium; however, these lesions exhibit different aggressive and biologic behaviors.[5] A large number of studies have focused on the stromal and epithelial



Graph 1: Comparison of expression of CD10 between odontogenic keratocyst and ameloblastoma.

Table 3: Comparison of AB and OKC with Fisher's exact test

Analysis test	*t*	*P*
Fisher's exact test	0.104	0.64

OKC: Odontogenic keratocyst; AB: Ameloblastoma

views of OKC (KCOT) and AB.[6-18] Researchers have been trying for many years to find ways to eliminate or restrict the development and spread of malignant tumors, with many studies focusing on stroma for finding therapeutic techniques.[19] Some studies have shown that an increase in the expression of CD10 in the stroma has a prominent role in the homeostasis, neoplastic changes, and tumor progression, with its apoptotic and proliferative properties in tumor cells, according to some studies.[9,10]

In 2011, Masloub *et al*. in Egypt reported the higher expression of CD10 in multicystic AB and lower expression of CD10 in dentigerous cysts and concluded that an increase in the expression of CD10 in dentigerous cysts indicates the neoplastic potential of dentigerous cysts and an increase in the aggression and local recurrence of AB.[6] This comparision have been done for CD10 expression in ameloblastomat that is high in multicystic AB in comparision with unicystic AB. 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. In addition, Oba *et al*.[8] showed that the expression of CD10 might be used as a marker for tumor progression in malignant melanoma, consistent with the results of the present study. According to previous studies and Table 2 in this study expression of CD10 is high in multicystic ameloblastoma and most of the okc is low as we know in many cases multicystic ameloblastoma is more progessive than okc.

In a study by Masloub *et al*.,[6] the staining for CD10 was high in different forms of MCA compared to UA and in UA compared to dentigerous cysts, consistent with the results of a study by Iezzi *et al*.,[14] who reported that such differences might be attributed to different biological properties of CD10, and more aggressive behavior of MCA, compared to UA and dentigerous cyst. The results showed membrane and cytoplasmic staining, especially on the surface of epithelial layers in dentigerous cysts and the researchers reported that compromise is an indication of the neoplastic potential of such cysts. In the present study, the expression of CD10 in the epithelial cell was observed as well, especially in the basal and parabasal layers of the epithelial layer of OKC (KCOT); So it might be indicated the neoplastic potential of KCOT epithelium and its tumor-like behavior, such as high rate of recurrence.

In this study the epithelial cells of UA samples did not show any positive staining for CD10 but in one sample of multicystic AB stellate reticulum showed cytoplasmic staining. These results is consisted with Aiad study[10] that showed cytoplasmic staining of the tumor cells in basal cell carcinoma (BCC) and SCC samples. Also in the study of Masloub *et al*.[6] cytoplasmic staining of multicystic AB was seen. Probably the present study is in agreement with the Ogawa study[21] in that the expression of CD10 was associated with neoplastic cell growth differentiation. Increased expression of this marker was associated with increasing tumor dysplasia.

A study by Piattelli *et al*.[22] showed that expression of CD10 in the stromal cells of oral SCC has a role in the poor prognosis, with an important relationship with metastasis to lymph nodes, local recurrence, and the histological grade; in this context, no relationship was detected with patient age, tumor size, and the clinical stage of the tumor. It is consisted of the results of a study by Makretsov *et al*.,[4] in which the stromal cells of normal breast and noninvasive ductal carcinoma did not exhibit expression of CD10. In contrast, high expression of CD10 was seen in samples metastasizing to axillary lymph nodes. In addition, a study by Ogawa *et al*.[21] 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*.[23] and Braham *et al*.,[24] 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.

Previous studies have reported a similar role for CD10 in compromising the prognosis and in recurrence and invasion.[4,15,21,22] In the present study, expression of CD10 in the ameloblastic stroma might indicate invasion and local progression of the tumor. Since a high rate of recurrence has been reported for OKC[3] and one of the pathologic mechanisms for OKC, in a manner similar to AB, is related to the matrix metalloproteinases,[25] it is possible that the expression of CD10 in OKC, similar to AB, be an indicator of the tumor's local invasion, making the name "tumor" suitable for OKC.

The future studies have been done with larger samples and other odontogenic cyst such as dentigerous cyst compared with OKC that's better these studies evaluated the correlation between expression of CD10and recurrence of OKC, Ameloblastoma.

CONCLUSION

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. It is suggested that the expression of CD10 marker be evaluated in larger sample sizes of OKCs and AB, and its expression be evaluated in other cysts and odontogenic tumors. Furthermore, patient follow-up is recommended, in association with the evaluation of the relationship between recurrence and expression of CD10 in OKC s and AB.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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