Original Article

A Comparative Study of Cytokeratin 7 Expression in Dentigerous Cyst and Radicular Cyst by Immunohistochemical Method

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Abstract

Background and Aim: It is difficult to identify the type of odontogenic cyst in some patients. Evidence shows that difference in expression level of different cytokeratins can be helpful for detection and identification of odontogenic cysts. The aim of this study was to investigate the cytokeratin 7 (CK7) expression in radicular cysts and dentigerous cysts.

Materials and Methods: In this cross-sectional study, 20 samples of radicular cysts and 20 samples of dentigerous cysts (10 inflammatory, 10 non-inflammatory) were selected from the archives of the Oral Pathology Department of Isfahan Dental School, which had been obtained by excisional biopsy. Age and gender of patients, and location of lesion were extracted from patient records. Immunohistochemical (IHC) staining for CK7 was performed and the expression level of this marker was determined based on the SID index. Data were statistically analyzed by the Mann-Whitney, Kruskal-Wallis, Chi-square, Fisher's exact, and t tests using SPSS version 24 at 0.05 level of significance.

Results: The two groups had no significant difference regarding the mean age (P=0.785), gender (P=0.490), or lesion location (P=0.172). There was a significant difference between radicular and dentigerous cysts in CK7 expression (P=0.003), and the pattern of staining (P=0.028). No significant difference was found between inflammatory and non-inflammatory dentigerous cysts based on CK7 expression (P=062).

Conclusion: Expression of CK7 may be useful for differentiation of dentigerous cyst from radicular cyst.

Key Words: Dentigerous Cyst; Radicular Cyst; Keratin-7

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Introduction

Odontogenic cysts are divided into two important categories of inflammatory and developmental cysts. Odontogenic cysts can cause symptoms such as tooth mobility, increased risk of jaw fracture in the affected area, pain, and swelling (1,2). Radicular cyst is one of the inflammatory odontogenic cysts that

accounts for 52% to 68% of all cystic lesions of the jaw. It originates from the remnants of the epithelial rests of Malassez as a result of an inflammatory process following dental caries and dental pulp necrosis. It is often associated with a non-vital tooth. Radiographically, it manifests as a radiolucent lesion in the root area that can cause root resorption. Histopathologically, a cystic cavity with a non-keratinized squamous epithelial lining is seen (3).

Dentigerous cyst is the second most common odontogenic cyst after radicular cyst (4). It is frequently associated with impacted third molars and accounts for 20% of all cystic lesions of the jaw. This cyst surrounds the crown of a tooth and is connected to the neck of unerupted teeth. Dentigerous cyst is formed by the accumulation of fluid between the reduced enamel epithelium and tooth crown, or between the layers of the reduced enamel epithelium. It occurs mainly in the second and third decades of life. Histopathologically, it manifests as a cystic cavity with a non-keratinized squamous epithelial lining without rete pegs (5). In some cases, infiltration of mild to severe chronic inflammatory cells, Russell bodies, and cholesterol clefts are also seen. Although most dentigerous cysts have a developmental origin, there are some cysts that appear to have an inflammatory pathogenesis (6,7).

Cytokeratins are a large and complex family of intermediate filament proteins in epithelial cells, consisting of at least 20 polypeptides. They are divided into acidic and basic groups. The expression of unique cytokeratins in the epithelium is closely related to the differentiation pathways of cells and tissues. The origin, morphological characteristics, and function of epithelial cells can be understood according to the variations in presence of cytokeratins (8). Cytokeratin 7 (CK7) is a low molecular weight cytokeratin and a type 2 intermediate filament protein which is found naturally in simple epithelium, transitional epithelium, and in secretory glands and blood vessels (9). Embryological investigations in mice and humans have shown the expression of CK7 specifically in the epithelium of the cervix, anorectum, esophagus-stomach area, and oral cavity (10).

Due to the existence of many similarities in different odontogenic cysts and the need to distinguish them from each other, various studies have been conducted to investigate the expression level of specific cytokeratins in different odontogenic cysts (11,12). Also, in many cases, the radiographic view is confusing for the diagnosis of lesions, and it can be difficult to distinguish between a radicular cyst and a dentigerous cyst especially during the mixed dentition period. Sometimes, due to the inflammatory nature of these cysts, it is not possible to reach a definitive diagnosis by histopathological examination alone, and specific diagnostic methods may be required. Thus, the aim of this study was to compare the expression of CK7 in dentigerous cysts and radicular cysts.

Materials and Methods

Case selection:

According to a statistical consultant, a total of 40 samples including 20 dentigerous cysts (10 inflammatory dentigerous cysts and 10 non-inflammatory dentigerous cysts) and 20 radicular cysts retrieved from the archives of the Oral Pathology Department of Isfahan Dental School were selected for this study after confirming the diagnosis of lesions and ensuring the optimal quality and adequacy of the tissue samples for IHC staining by two oral pathologists. Age, gender, and location of lesion were extracted from patient records. Samples without the required clinical information, inappropriate histological quality, or tissue inadequacy were excluded from the study. Also, only the samples that were obtained by excisional biopsy were included in the study.

IHC staining:

Expression of CK7 was determined immunohistochemically by the standard biotin-streptavidin method. Histological sections were cut from paraffin embedded blocks with 3-4 μm thickness, and mounted on poly-L-lysine-coated slides. For antigen retrieval, tissue sections were deparaffinized and rehydrated with distilled water. Then,

sections were placed in 3% H2O2 for 3 minutes and were rinsed under running tap water. Antigen retrieval was carried out by heating 1,500 mL of the recommended retrieval solution (0.01 M citrate buffer, pH 6.0) until boiling in a pressure cooker. It was then kept for 10 minutes. The sections were subsequently placed in cold water and washed in Tris buffer saline for 15 minutes and diluted in saline for 10 minutes. The sections were incubated with CK7 primary antibodies (mouse monoclonal, clone OV-TL 12/30, 1:100 dilution; DAKO) overnight at 4°C (Dako En Vision FLEX system; Dako, Glostrup, Denmark) and washed in Tris buffer saline for 2-5 minutes and were later incubated in appropriate biotinylated secondary antibody for 1 hour at room temperature (mouse En Vision System HRP, Dako Cytomation). Visualization was performed using freshly prepared di-amino-benzidine chromogen for 10 minutes and the slides were counter-stained with the hematoxylin stain (Merck KGaA, Darmstadt, Germany). Appropriate positive and negative controls were also used.

Assessment of IHC staining:

For IHC staining, all slides were evaluated by two oral pathologists in a blinded manner under a light microscope (BX41TF; Olympus, Tokyo, Japan). The nuclear or/and cytoplasmic expression of CK7 was evaluated. The epithelial cells were evaluated using a semi-quantitative scale: 0 (negative: without immuno-stained cells), +1 (1% to 25% immuno-stained cells), +2 (26% to 50%), +3 (51% to 75%) and +4 (>75%). Furthermore, staining intensity was scored using the following scale: 0 (no immuno-stained cells), +1 (very low staining), +2 (moderate), +3 (moderate to high), and +4 (high staining). Staining intensity distribution (SID) score was calculated by multiplying the distribution by staining intensity (13). The staining pattern was categorized as focal and uniform. Also, staining in the superficial, suprabasal and spinous layers was investigated (14).

Statistical analysis:

All clinical, histopathological, and immunohisto-chemical data were analyzed by SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) to assess statistically significant differences between the study groups using the Mann-Whitney, Kruskal-Wallis, Chi-square, Fisher's exact and t tests. A P-value <0.05 was considered statistically significant.

Results

Clinical analysis:

Table 1 shows the frequency distribution of the groups based on age, gender, and location. The mean age of patients was 27.8±16.23 years in the dentigerous cyst group and 33.4 ± 15.47 years in the radicular cyst group; the difference between the two groups was not significant in this regard based on t-test (P=0.785). But, the mean age of patients showed a significant difference between inflammatory and non-inflammatory dentigerous cysts (P=0.006).

According to the Chi-square test, there was no significant difference between dentigerous and radicular cyst groups based on gender of patients. Males were more commonly affected by non-inflammatory dentigerous cyst than females, while the number of males and females was almost the same in inflammatory dentigerous cyst group, and there was no significant difference between the two groups in this regard (P=0.350).

The majority of the samples were found in the posterior region of the mandible, and Fisher's exact test did not show a significant difference between dentigerous cyst and radicular cyst in this respect (P=0.490). Also, no significant difference was found between inflammatory and non-inflammatory dentigerous cysts in this regard (P=1.000).

IHC analysis:

Table 2 shows the frequency distribution of the groups based on IHC analysis. The expression of CK7 was evaluated using the frequency and intensity of stained cells reported based on the SID index. According to the results, CK7 expression was higher in radicular cysts than dentigerous cysts and this difference was significant according to t-test (P=0.003). But, CK7 expression did not show a significant difference between inflammatory and non-inflammatory dentigerous cysts (P=0.062),

Table 1. Clinical characteristics of the study groups

Variables \ Groups		Dentigerous cyst		Radicular cyst	P value
		Inflammatory	Non-inflammatory		
Age (mean± SD)		20.6 ± 8.93	35 ± 19	33.4 ± 15.47	0.785
Gender	Male	5 (50%)	8 (80%)	15 (75%)	0.490
	Female	5 (50%)	2 (20%)	5 (25%)	
Location	Anterior maxilla	2 (20%)	2 (20%)	3 (15%)	0.172
	Posterior maxilla	1 (10%)	0 (0%)	6 (30%)	
	Anterior mandible	0 (0%)	0 (0%)	0 (0%)	
	Posterior mandible	7 (70%)	8 (80%)	11 (55%)	

SD: Standard deviation

Table 2. Immunohistochemical parameters of the study groups

CK7 \ Groups		Dentigerous cyst		Radicular cyst	P value
		Inflammatory	Non-inflammatory		
SID index (mean± SD)		5.00 ± 2.21	4.80 ± 3.15	7.40 ± 4.32	0.003
Pattern	Focal	4 (40%)	4 (40%)	2 (10%)	0.028
	Uniform	6 (60%)	6 (60%)	18 (90%)	
Epithelial layer	Superficial	4 (40%)	8 (80%)	8 (40%)	0.206
	Suprabasal and spinous	6 (60%)	2 (20%)	12 (60%)	

SD: Standard deviation

although expression of CK7 was slightly higher in inflammatory dentigerous cysts.

The pattern of staining in most lesions was uniform. Based on the Chi-square test, there was a significant difference in the staining pattern between dentigerous cyst and radicular cyst (P=0.028), but this difference was not significant based on the Fisher's exact test between the inflammatory and non-inflammatory dentigerous cysts (P=1.000).

According to the Chi-square test, no significant difference was found between the dentigerous cyst and radicular cyst based on the stained epithelial layer (P=0.206). There was no significant difference between the inflammatory and non-inflammatory dentigerous cysts according to the Fisher's exact test (P=0.172, Figure 1).

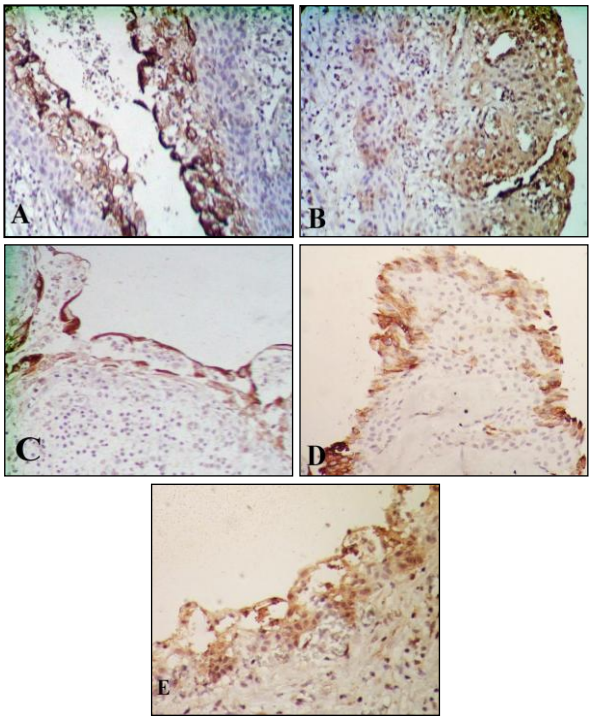

Figure 1. (A) Uniform pattern of CK7 expression in a radicular cyst, (B) Ck7 expression in superficial layers with moderate staining intensity in a dentigerous cyst, (C) focal expression with low staining intensity in a radicular cyst, (D) high intensity of staining in a radicular cyst, (E) moderate staining intensity in the suprabasal and spinous layers of a dentigerous cyst (IHC staining, ×400)

Discussion

Tooth development is a complex and highly coordinated process with a number of successive morphological stages. Temporal changes in the profile of cytokeratins during odontogenesis have been investigated in different studies. The odontogenic epithelium in normal tissue expresses cytokeratins 14, 13, 7 and 19. These cytokeratins can be expressed in different proportions based on the degree of differentiation of epithelial cells in odontogenic lesions (15). CK7 is a typical filament of stellate reticulum and Hertwig's epithelial root sheath cells (16). In the present study, all radicular cysts expressed CK7, which was in line with the study by Hornia et al, (17) while 41.66% of radicular cysts in a study by Saluja et al, (14) 26.7% in a study by Stoll et al, (18), 40% in a study by Gao et al, (19) and only 4% in a study by Matthews et al. (20) were positive for CK7. Contrary to the present study that all dentigerous cysts expressed CK7, 66.66% in the study by Saluja et al, (14), 76.66% in the study by Stoll et al, (18) and 70% of dentigerous cysts in a study by Pires et al. (21) were positive for CK7. Also, in a study by Gratzinger et al, (22) two samples of dentigerous cysts were reported to be negative for this marker. Furthermore, in studies by Sadiq et al, (23) Pires et al, (21) and Gratzinger et al, (22) all odontogenic glandular cysts expressed CK7. The difference in the staining rate of the lesions may be due to the type of antibody used and the adopted IHC technique. In the present study, the expression of CK7 by radicular cysts was significantly higher than dentigerous cysts, which is contrary to the results of the studies by Saluja et al, (14) and Stoll et al (18). In the present study, CK7 expression in inflammatory dentigerous cysts was observed more than in non-inflammatory type. In the present study, a larger sample volume was examined than other studies and also the SID index was used, which is an accurate and reliable measure to evaluate the frequency and intensity of staining of cells in IHC samples, which may be responsible for the difference between the results of the present study and other studies. According to the results of the present study, it appears that CK7 expression increases by increasing the level of inflammation of the lesion but more studies in this regard are required.

In the present study, 90% of radicular cysts showed a uniform staining pattern for CK7, while 60% of dentigerous cysts had this staining pattern, and a significant difference was found between them. On the other hand, the staining pattern did not differ between inflammatory and non-inflammatory dentigerous cysts. However, in the study by Saluja et al, (14) 100% of radicular cysts had a focal staining pattern and 60% of dentigerous

cysts had a uniform staining pattern. In the present study, 60% of radicular cysts had CK7 expression in the suprabasal layers, and 60% of dentigerous cysts had CK7 expression in the superficial epithelial layers, although this difference was not significant. Also, 60% of inflammatory dentigerous cysts had staining of suprabasal cells and 80% of non-inflammatory dentigerous cysts had staining of superficial layers. In the study by Saluja et al, (14) most of the radicular cysts had CK7 expression in the superficial layers, which was in contrast to the present results. Consistent with the present study, Stoll et al. (18) showed that most of the radicular cysts had CK7 expression in the suprabasal layers, although in their study, most dentigerous cysts also showed this pattern (18). The difference in IHC staining techniques and the type of antibody used can be the reasons for the difference in the results of the studies, but more studies are still needed.

In the present study, consistent with other studies, the mean age of patients with dentigerous cysts was lower than the mean age of patients with radicular cysts (23,24). Also, the majority of the studied samples were observed in males compared with females and the posterior region of the mandible was more commonly involved than other areas, like other studies (23,25,26)

Conclusion

Considering the higher expression of CK7 in radicular cysts compared to dentigerous cysts, this marker may be used to differentiate these two cysts from each other. Although other studies with larger sample size are suggested.

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