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Original Article

Expression of Bcl-2 and epithelial growth factor receptor proteins in keratocystic odontogenic tumor in comparison with dentigerous cyst and ameloblastoma

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ABSTRACT

Background: Keratocystic odontogenic tumor (KCOT) is a developmental odontogenic cyst on which various investigations have been focused due to its biological activities, high tendency to recur and different growth mechanisms in comparison with other cystic lesions. Previous studies have shown different biological and proliferative activities for the lining epithelium of KCOT. The aim of this study was immunohistochemical evaluation of Bcl-2 and epidermal growth factor receptor (EGFR) expression in KCOT compared with dentigerous cyst and ameloblastoma.

Materials and Methods: Formalin-fixed and paraffin-embedded tissue sections of 16 cases of KCOT, 16 cases of dentigerous cyst and 16 cases of ameloblastoma were immunohistochemically analyzed to determine Bcl-2 and EGFR proteins' expression. Biotin-Stereotavidin method was used. It was observed by two oral pathologists separately, and the data were analyzed by Mann–Whitney and Kruskul–Wallis. $P < 0.05$ was considered as significant.

Results: Regardless of staining intensity, all cases of ameloblastoma and KCOT except dentigerous cases were positively stained for Bcl-2. Expression of Bcl-2 was higher in the peripheral layer of ameloblastoma and basal layer of KCOT. Furthermore, all cases of ameloblastoma and dentigerous cysts except KCOT samples were positively stained for EGFR. Expression of EGFR was higher in the peripheral layer of ameloblastoma and basal layer of dentigerous cysts.

Conclusion: According to the expression of — Bcl-2 in ameloblastoma and KCOT, and no expression of EGFR in KCOT, it can be concluded that the biological activity and growth mechanisms of KCOT are different compared with other cystic lesions. However, the aggressive potential of KCOT is not as severe as that of a neoplasm such as ameloblastoma.

Key Words: Ameloblastoma, Bcl-2 protein, dentigerous cyst, epiderm, growth factor, epidermal growth factor receptor, immunohistochemistry, odontogenic tumor

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INTRODUCTION

Keratocystic odontogenic tumor (KCOT) is a developmental odontogenic cyst. Various investigations have focused on it due to its biological activities, high tendency to recur and different growth mechanisms in comparison with other cystic lesions.[1,2] KCOT may have a neoplastic potency. Therefore, it has an aggressive clinical behavior.[3-9]

Many studies have been conducted on cell-cycle associated proteins such as antiapoptotic markers (tp53, Bcl-2) and cell proliferation markers (ki67, proliferating cell nuclear antigen, epidermal growth factor receptor [EGFR]).[10-13] These studies have

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shown different biological and proliferative activities for the lining epithelium of KCOT.[4,14,15]

Bcl-2, which is located in the external membrane of mitochondria, endoplasmic reticulum and nuclear membrane, play an important role in apoptosis.[16] Overexpression of Bcl-2 in tumoral cells causes apoptotic resistance and increase of cell growth.[16] Uncontrolled expression of Bcl-2 has been found before histopathologic changes in the early stage of neoplasm.[17,18]

Epidermal growth factor receptor is the most important growth factor ligand on the cell surface. Overexpression of EGFR-related genes is seen in many neoplasms, which causes the over sensitivity of cells to the normal level of growth factor. Nowadays, EGFR is known as an effective growth factor in many human cancers.[16] EGFER signaling is associated with malignancy transformation. Therefore, it causes specific phenotypes of cells, which can affect the cellular reaction to the treatment.[19]

Kichi *et al*. have reported that Bcl-2 is seen only in KCOT and not in dentigerous cyst.[12] Sandra *et al*. have reported strong expression of Bcl-2 in ameloblastoma and suggested that antiapoptotic proteins are expressed more than apoptotic proteins in ameloblastoma.[20] In a study done by Shear, expression of EGFR marker was reported in the epithelium of KCOT, dentigerous and radicular cysts. The strongest reaction was related to KCOT, and the weakest was in the radicular cyst.[3]

Indeed, because of specific activity of KCOT in comparison with other odontogenic cysts, it is possible to evaluate the expression of EGFR and Bcl-2 proteins in KCOT compared with dentigerous cyst and ameloblastoma. Expression of these proteins provides us with the knowledge about the biological activity of KCOT. In this study, the expression of EGFR and Bcl-2 proteins in KCOT was evaluated and compared with dentigerous cyst and ameloblastoma.

MATERIALS AND METHODS

In a cross-sectional study, paraffin-embedded tissues of 16 KCOT, 16 dentigerous cysts, and 16 ameloblastoma samples were obtained from the archives in Department of Oral Pathology, Dental School, Isfahan University of Medical Sciences.

In order to detect the specific antigens of EGFR and Bcl-2, the tissues were immunohistochemically stained by Biotin-Stereotavidin method. Briefly, the main procedures were: Serial sectioning (in 3-4 um sections), deparafinization, rehydration, and antigen retrieval.

All specimens were then placed in the phosphate-buffered saline (PBS) and treated for 5 min in protein block solution to prevent any false staining. The specimens were then incubated for 30 min with primary antibody of EGFR (NCL-EGFR-384) clone EGFR25, Bcl-2 (NCL-BCL-2-486) clone 3.1 (Novocastra/Germany).

Furthermore, the sections were exposed to Novolink polymer (RE7112) or a secondary antibody for 30 min and washed with PBS. They were then incubated for 5 min with diaminobenzidine for visualization. After washing the slides, they were counter stained with hematoxylin. Finally, the slides were mounted after being dried.

To quantify the percentage of positive cells within the lesions of EGFR and Bcl-2 markers, the sections were observed by two oral pathologists separately by Olympus light microscope (CX21FS, Tokyo, Japan) at ×400 magnification.

The score of EGFR staining and Bcl-2 in each specimen/layer was calculated by the following formulas:

- Score of EGFR staining in each layer = D × I
- Score of EGFR staining in each specimen = TD × TI
- Distribution (D): Mean percentage of stained cells by 1000 cells in each layer
- Intensity of staining in each layer (I): Negative (0), weak (+1), moderate (+2), severe (+3)
- Pattern of staining in each specimen (P): Membranous, cytoplasmic, membranous — cytoplasmic
- Total distribution of each (TD): Mean distribution of the total layer
- Total intensity (TI): Mean intensity of total layer.

According to the distribution, the intensity of Bcl-2 staining was classified as below:

D/TD intensity <%1:0/10-25%:+1/25-50%:+2/>50%:+3.

Data were statistically analyzed by Mann–Whitney and Kruskul–Wallis tests using SPSS software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as significant.

RESULTS

The results of immunohistochemical staining of EGFR marker showed that none of the KCOT specimens expressed EGFR with the score of 0. The score of ameloblastoma specimens was 1.54. Dentigerous cysts also indicated the score of 1.2 [Figures 1 and 2, Table 1]. There was no difference between the intensity of EGFR expression in ameloblastoma and dentigerous cyst specimens. On the other hand, the distribution of this marker was significantly different. 91.8% of cells in ameloblastoma and 74.8% of cells in dentigerous cyst expressed this protein. The pattern of expression in 94% of ameloblastoma specimens was membranous, and it was membranous-cytoplasmic in 100% of dentigerous cysts specimens. There was a significant difference between EGFR expression in peripheral and internal layers of ameloblastoma. 98.4% of the peripheral layer cells and 85.3% of the internal layer cells expressed EGFR protein. The intensity of EGFR expression was also significantly different in peripheral and internal layers ($P = 0.01$). In the evaluation of different layers of dentigerous cysts, the scores of EGFR expression in basal cells versus supra-basal and superficial layers were 1.85 and 0.47, respectively.

The results of immunohistochemical evaluation of Bcl-2 marker revealed that the intensity of Bcl-2 expression was not significantly different in ameloblastoma and KCOT. The intensity of Bcl-2 expression in ameloblastoma showed statistically significant differences compared with dentigerous cyst [Figures 3 and 4, Table 2].

DISCUSSION

Expression of Bcl-2 protein with high intensity in all of the ameloblastoma specimens (as a neoplastic lesion) and KCOT (as a cystic lesion with neoplastic features) indicated the abnormal control of cell-cycle in these two lesions. This result can be due to the increase of cell vitality and invasive growth pattern of the lesion, which is similar to those reported by Lo Muzio *et al*., Kichi *et al*. and Piattelli *et al*.[4,12,14]

Strong expression of Bcl-2 in the peripheral layers and minimal expression in the central layers of ameloblastoma indicated the high potential of cell vitality in peripheral layers. It can be argued that

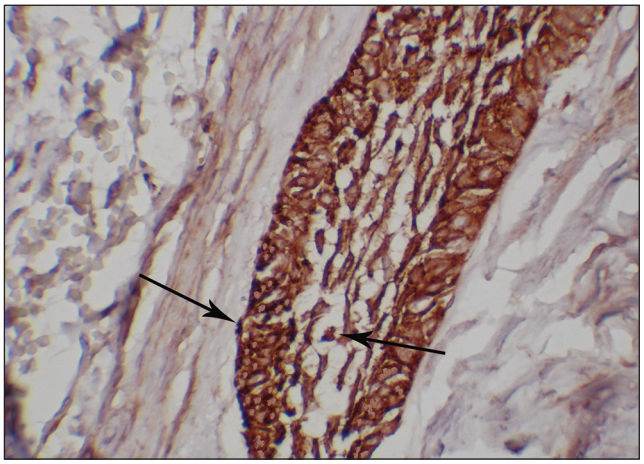

Figure 1: Epidermal growth factor receptor expression in ameloblstoma.

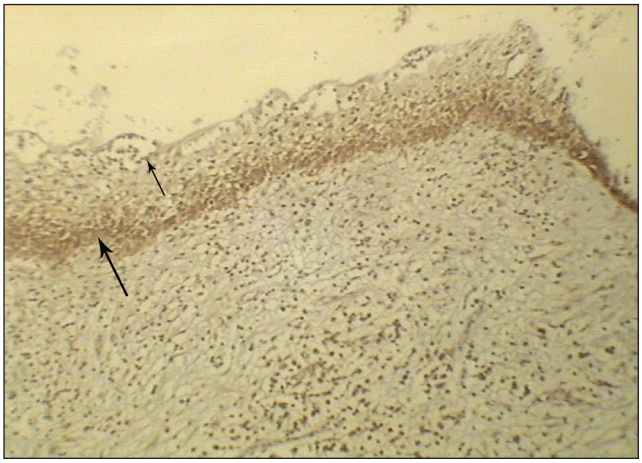

Figure 2: Epidermal growth factor receptor expression in dentigerous cyct.

Table 1: EGFR marker expression in ameloblastoma, KCOT and dentigerous cyst ($P < 0.01$)

Lesion	Intensity									
	0		1 (weak)		2 (moderate)		3 (severe)		Total	
	Distribution	(%)	Distribution	(%)	Distribution	(%)	Distribution	(%)	Distribution	(%)
Ameloblastoma	0	0	5	31.2	11	68.8	0	0	16	100
KCOT	16	100	0	0	0	0	0	0	16	100
Dentigerous cyst	0	0	6	37.5	10	62.5	0	0	16	100

KCOT: Keratocystic odontogenic tumor; EGFR: Epidermal growth factor receptor

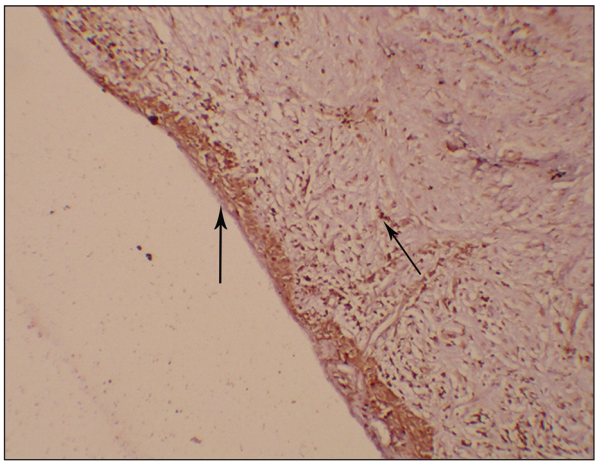

Figure 3: Bcl-2 expression in dentygerous cyst.

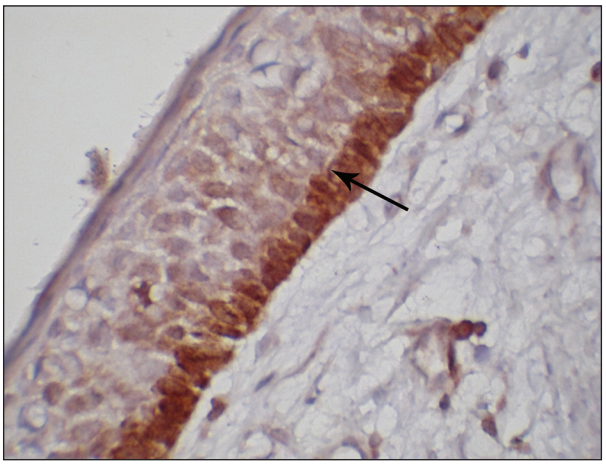

Figure 4: Bcl-2 expression in keratocystic odontogenic tumor.

Table 2: Bcl-2 marker expression in ameloblastoma, KCOT and dentigerous cyst ($P = 0.02$)

Lesion	Intensity									
	Negative		+1		+2		+3		Total	
	Distribution	(%)	Distribution	(%)	Distribution	(%)	Distribution	(%)	Distribution	(%)
Ameloblastoma	0	0	1	6.3	9	56.3	6	37.5	16	100
KCOT	0	0	0	0	16	100	0	0	16	100
Dentigerous cyst	16	100	0	0	0	0	0	0	16	100

KCOT: Keratocystic odontogenic tumor

Bcl-2 has an important role in the survival of main cells in the peripheral layers of ameloblastoma. Higher expression of Bcl-2 in the basal layer of KCOT is related to the physiological potency of the basal layer as a cell source for epithelium.

According to the present results, no expression of EGFR (as the most important protein in neoplastic proliferation) was observed in KCOT. No expression of this protein and high level of Bcl-2 protein expression show that aggressive features of KCOT are related to apoptotic proteins. However, the aggressive potential of KCOT is not as severe as that of a neoplasm such as ameloblastoma. Indeed, the significant expression of antiapoptotic proteins with lack of proliferative proteins can cause a neoplastic condition with less aggressive features.[16,21]

High and severe expression of EGFR and Bcl-2 in ameloblastoma shows the neoplastic nature of this lesion. Bcl-2 expression can cause an increase in the cell vitality. On the other hand, EGFR expression causes an increase in the proliferation and aggressive activity of this lesion.

Ameloblastoma can infiltrate into the bone's trabeculas. Therefore, its treatment needs aggressive surgical techniques. In aggressive ameloblastoma cases, control of proliferation and cell vitality can be effective in the treatment and recurrence.[19,22] The goal of investigations is to find less aggressive methods such as a monoclonal antibody of anti-EGFR protein to prevent functional damage of the chewing system and deformity caused by surgical procedures. Anti-EGFR drugs and radiotherapy have synergistic effects in controlling the neoplasm and decreasing the recurrence.[23-25]

In physiological conditions, EGFR has a membranous and cytoplasmic expression.[26] All cases of dentigerous cyst indicated a membranous-cytoplasmic expression of EGFR with lower value and distribution than ameloblastoma. Kichi *et al*. have reported the high distribution of TdT-mediated dUTP-biotin nick end labeling positive cells in the superficial layers and lack of these cells in the basal and middle layers of dentigerous cyst. However, these findings show apoptosis in the superficial layer of dentigerous cyst.[12] Furthermore, in ameloblastoma, the peripheral layers are proliferative components.[27]

More severe expression and higher distribution of EGFR protein in the peripheral layer of ameloblastoma have the same pattern as Bcl-2 expression compared with the central layer. Li *et al*. have reported that the proliferative nature of cells decreases from the peripheral layer to the central layer of tumoral mass.[15]

CONCLUSION

Biological activities, high tendency to recur and growth mechanisms of KCOT are different in comparison with other cystic lesions that are related to apoptotic proteins. However, the aggressive potential of KCOT is not as severe as that of the neoplasms such as ameloblastoma.

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