

Expression of bcl-2 in the Epithelial Lining of Odontogenic Keratocysts

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Abstract:

Statement of Problem: The aggressive nature and high recurrence rate of Odontogenic Keratocysts (OKCs) may be due to unknown factors inherent in the epithelium or because of enzymatic activity in the fibrous wall. Bcl-2 protein is characterized by its ability to inhibit apoptosis.

Purpose: The aim of the present study was to analyze the expression of bcl-2 protein in OKCs and to compare it with the more common radicular and dentigerous cysts. The possible relationship between inflammation and bcl-2 expression was also investigated.

Materials and Methods: Formalin fixed paraffin-embedded tissue sections of 20 OKCs, 20 radicular and 20 dentigerous cysts were immunohistochemically analyzed for immunoreactivity of the bcl-2 protein.

Results: Bcl-2 expression was observed in 19 OKCs (95%), one radicular cyst (5%) and one dentigerous cyst (5%). There was no statistically significant relationship between inflammation and the number of bcl-2 positive cells. Immunoreactivity was mainly noted in the basal or basal/supra basal layers.

Conclusion: Considering the fact that bcl-2 over expression may lead to increased survival of epithelial cells, present study may demonstrate a possible relationship between the aggressive nature of OKC and the intrinsic growth potential of its lining epithelium. Furthermore a basal/supra basal distribution of bcl-2 positive cells was seen in some odontogenic keratocysts which may have a significant impact on the behavior of this cyst.

Key Words: Bcl-2; Odontogenic cysts; Odontogenic keratocyst; Radicular cyst; Dentigerous cyst; Immunohistochemistry

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INTRODUCTION

The odontogenic keratocysts (OKC) demonstrate a high recurrence rate and a biologically aggressive nature. This cyst has been found to be the third most common cyst in Isfahan province (central Iran), after radicular and dentigerous cysts [1]. The reported frequency of recurrence in various studies ranges from 5% to 60% [1-3]. Some mechanisms have

been suggested to explain its behavior and high tendency for recurrence, such as the difficulty to remove it in one piece due to the thin, friable nature of the cyst wall, satellite small daughter cysts, production of bone resorptive factors in the cyst wall and increased proliferation of the epithelial lining of the cyst [4]. Several studies have been conducted on proteins involved in cell cycle

division, such as P53, Ki-67 and bcl-2. Comparing OKC with other odontogenic cysts, Slootweg [5] showed an increased expression of P53 and Ki-67 in the epithelial lining of OKC as compared to other cysts. Ki-67 is known as a cell proliferation marker and P53 acts as a cell cycle regulator. The bcl-2 family is recognized as a cell division regulator, which can inhibit apoptosis and produce extended cell survival [6]. Bcl-2 proteins have been identified in many tumors [6–14]. In 1998, Piatelli [15] in his sample of 53 odontogenic cysts, found all OKCs to be positive for bcl-2 while the studied dentigerous and radicular cysts were negative. Furthermore in 1999, Muzio et al [16] demonstrated positivity of bcl-2 in 13 of the 16 studied sporadic OKCs as compared to 15 of the 16 syndromic OKCs. The aim of the present study was to evaluate bcl-2 expression and distribution in the epithelial lining of non-syndromic odontogenic keratocysts and its possible relationship with inflammation. A comparison of bcl-2 expression was also carried out between OKC, dentigerous and radicular cysts.

MATERIALS AND METHODS

Sixty formalin fixed paraffin embedded blocks of OKCs, dentigerous and radicular cysts (20 of each) were retrieved from the archive of the Department of Oral Pathology, School of Dentistry, Isfahan University of Medical Sciences, by random sampling and were evaluated for the expression of bcl-2. None of the OKCs were associated with Nevoid basal cell carcinoma syndrome (NBCS). Consecutive sections were employed as negative controls for immunohistochemical analysis and for H&E staining to confirm the diagnosis. 4µm thick sections were cut and mounted on poly-L-lysine-coated slides, air dried and heated at 45°C overnight. After deparaffinization and rehydration, the sections were incubated in 0.01 M citrate buffer in a

microwave oven for 15 min for antigen retrieval. The slides were then washed in phosphate-buffered saline (PBS) for 30 min at room temperature and incubated in 0.5% H₂O₂ in methanol for 10 min to block endogenous peroxidase activity. Nonspecific antibody binding was blocked with 3% normal horse serum in PBS. A mouse anti-human bcl-2 monoclonal antibody (Clone: Bcl-2-100, Zymed, South San Francisco, CA, USA) with a 1/100 dilution was applied as primary antibody and placed in a humidified chamber at 4°C overnight. The sections were subsequently washed in PBS and processed for detection of the positive immunohistochemical reaction using the streptavidin-biotin complex immunoperoxidase technique. Rabbit-mouse antibody link solution (Zymed ABL008, South San Francisco, CA, USA) was used as secondary antibody to enhance the sensitivity of the procedure and diaminobenzidine was applied as the chromogen. Sections were finally counterstained with hematoxylin, cleared and mounted. Negative controls consisted of phosphate buffered saline instead of primary antibody and a sample of lymphoma and inflammatory cells within the cyst wall were used as the external and internal positive controls. Several slides were selected randomly for reevaluation by either one of the researchers who were blinded to the previous results (intra-observer and inter-observer validity). All stained areas demonstrating positivity for bcl-2 were identified at a magnification of ×40 and the number of positively stained cells was counted on 10 representative areas of the epithelium using a ×40 objective in a minimum of 100 cells in the full length of the epithelium. The intensity of bcl-2 positivity was estimated as previously described [16]: (-) fewer than 5% positive cells or no staining; (±) 5% to 9% positive; (+) 10% to 24% positive; (++) 25% to 50% positive; and (+++) more than 50% positive. The degree of inflammation was assessed in the

connective tissue stroma adjacent to the epithelial lining in which the cell count had been performed (magnification, ×100) and also approximal to bcl-2 negative cases. A lack of inflammatory cells was scored as (-), less than 30 inflammatory cells as (+), 30 to 59 inflammatory cells as (++) and 60 or more inflammatory cells as (+++).

Collected data were analyzed using the SPSS software for Windows (Version 10.0). Inter-observer agreement was measured by Kappa. In cases where the observers had reported two different intensity values, the higher one was considered accurate. Data analysis was performed using Chi-square, Kruskal-Wallis, Mann-Whitney and Spearman correlation coefficient, with the level of significance set at $p < 0.05$.

RESULTS

Staining intensity for bcl-2 in the studied odontogenic cysts are shown in Table I. Significant difference was observed in staining intensity between OKCs, dentigerous and radicular cysts, (Kruskal-Wallis, $P < 0.001$). In multiple comparisons, bcl-2 staining intensity revealed a statistically significant difference between OKCs and radicular cysts and between OKCs and dentigerous cysts (Mann-Whitney, $P < 0.001$). There was no significant

Table I: The frequency of Immunohistochemical expression of bcl-2 in odontogenic cysts.

Cyst type	Intensity of Expression				
	—	±	+	++	+++
Odontogenic Keratocyst	1 (5%)	-	5(25%)	13(65%)	1(5%)
Dentigerous cyst	18(90%)	1(5%)	1(5%)	-	-
Radicular cyst	17(85%)	2(10%)	-	-	1(5%)

Bcl-2 positive cells (×100): Less than 5% (-); 5-9% (±); 10-24% (+); 25-50% (++); Over 50% (+++).

Table II: Intensity of inflammation in odontogenic cysts

Type of Cyst	Intensity of inflammation			
	±	+	++	+++
Odontogenic Keratocyst	3(15%)	5 (25%)	3(15%)	9 (45%)
Dentigerous cyst	3(15%)	3(15%)	1(5%)	13(65%)
Radicular cyst	-	4(20%)	3(15%)	13(65%)

Intensity of inflammation (×100): No Inflammatory cells (-); Less than 30% inflammatory cells (+); 30-59% of cells (++); 60% and more cells (+++)

difference between radicular and dentigerous cysts (Mann-Whitney, $P = 0.6$).

Positive bcl-2 staining was considered when at least 10% of the cells were stained. Nineteen out of 20 OKCs (Fig. 1), one out of 20 dentigerous cysts (Fig. 2) and one out of 20 radicular cysts (Fig. 3) were positive for bcl-2 and revealed a significant difference (chi-square, $P < 0.001$).

In 11 of the 19 stained OKCs, positively stained cells were observed in the basal layer (Fig. 1) while in the other bcl-2 positive OKCs (8 of 19), the stained cells were in the basal/supra basal region (Fig. 4). In bcl-2 positive radicular and dentigerous cysts, positive cells were located in the basal/supra basal layers.

Inflammation intensity in the cyst walls are shown in Table II. No statistically significant relationship was found between the intensity of inflammation and bcl-2 staining in positively-stained OKCs (Spearman = 0.06, $P = 0.8$) and the other two cyst types.

The sensitivity, specificity and positive and negative predictive values of bcl-2 staining for the differentiation of OKC from the other studied cysts were 95%, 90%, 90% and 97% respectively, with acceptable inter-observer agreement (Kappa = 0.85).

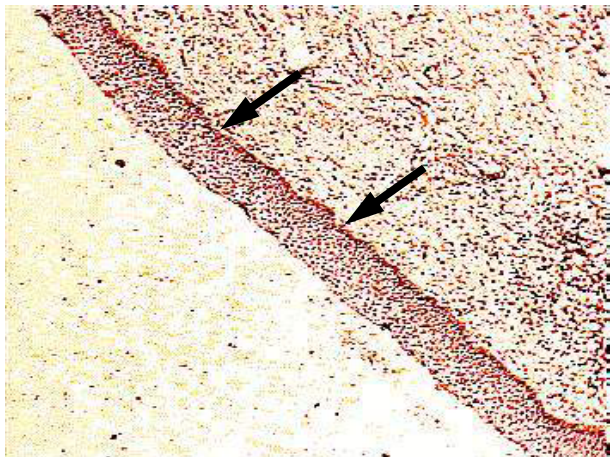


Fig.1: Odontogenic keratocyst. Most of the basal cells are positive for bcl-2 (X100).

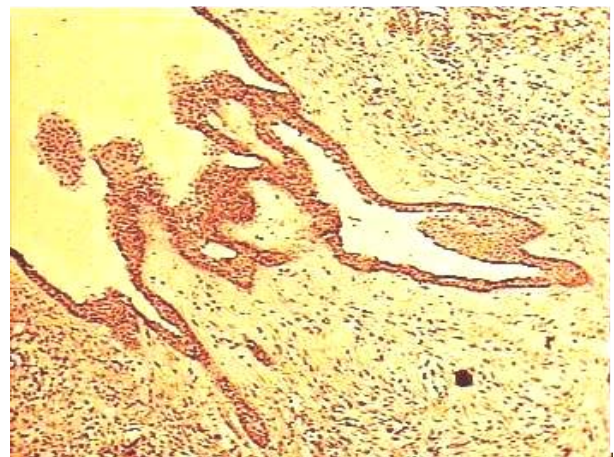


Fig.2: Dentigerous cyst. Basal and Suprabasal cells are positive for bcl-2. (X100).

DISCUSSION

In the present study, the epithelial lining of 19 out of 20 OKCs was positive for bcl-2, while only 1 out of 20 dentigerous cysts and 1 out of 20 radicular cysts revealed positivity.

Our findings are in agreement with the results of a study conducted by Piattelli et al (1997) (15) who found bcl-2 positivity in all 14 studied OKCs (12 parakeratinized and 2 orthokeratinized, with similar staining patterns), 1 of the 19 dentigerous cysts and none of the 20 radicular cysts. All the OKCs in the present study were parakeratinized and similar to Piattelli's report, cases with a minimum staining of 10% were considered

positive. Muzio, et al [16] found bcl-2 positivity in 13 of 16 solitary and 15 of 16 syndrome associated (NBCCS) OKCs. None of our cases were syndromic but these findings are also in accordance with our results.

In 2000, Tosios et al [17] studied the expression of Ki-67, P₅₃ and bcl-2, in dentigerous cysts. They found weak positive staining in the basal layer of 1 of the 6 cases and strong positive staining in the basal and suprabasal layer in another one. In the current study, one dentigerous and one radicular cyst showed positive bcl-2 staining in the basal and suprabasal cells. Muzio et al [16] reported the bcl-2 staining area in OKCs to be restricted to

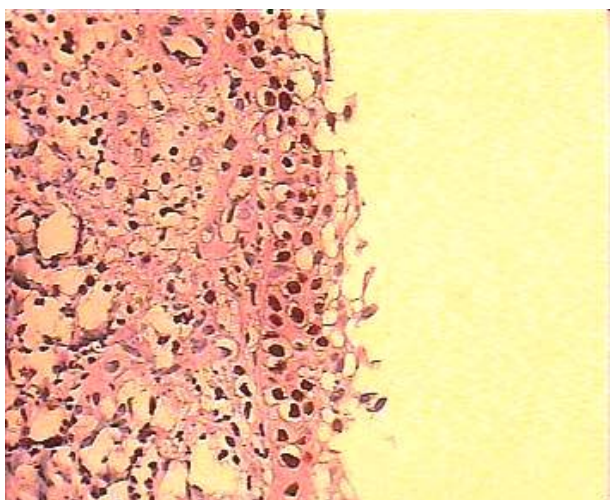


Fig. 3: Radicular cyst. Basal and Suprabasal cells are positive for bcl-2. (X400).

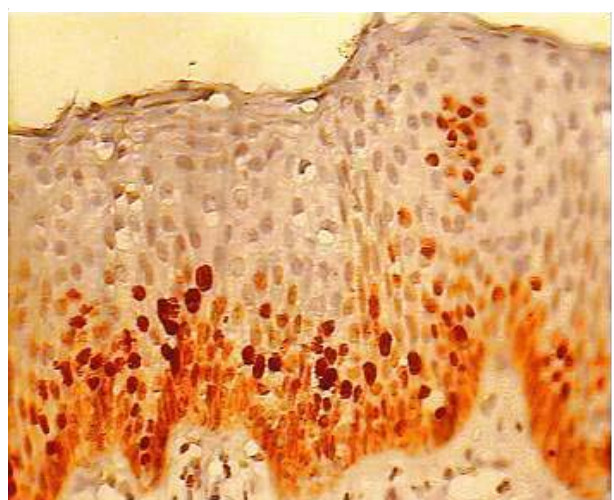


Fig. 4: Odontogenic Keratocyst. Basal and Suprabasal cells are positive for bcl-2. (X400).

the basal layer, but Piatteli et al [15] demonstrated positivity in the basal and supra basal region of the epithelial lining. Eleven and 8 of the 19 OKCs in the present study showed positivity in the basal layer and basal/supra basal layers, respectively.

Since, the basal layer of normal mucosal epithelium is also positive for bcl-2 [18,19]; the lack of expression in the upper layers may be due to a decrease in the dividing ability and termination of the cell's life span.

The two groups of OKCs with different bcl-2 distribution patterns were compared in order to evaluate the possible relationship between staining of the upper layers of the epithelium and increased recurrence.

In Piatteli's study, bcl-2 immunoreactivity decreased significantly in the presence of inflammation [15]. Inflammation in OKC may change the morphologic appearance of the epithelial lining or it may in some way alter the proliferative potential of the cells. The present study was unable to demonstrate a significant relationship between the degree of inflammation and bcl-2 immunoreactivity, which may be due to an inadequate sample size or an improper measuring method.

Concerning the diagnostic value of bcl-2 staining in demarcation of OKCs from other odontogenic cysts; sensitivity, specificity and positive and negative predictive values were 95%, 90%, 90%, and 97% respectively. These findings were in accordance with Piatteli's study [15] which corresponding values were reported as 100%, 97%, 93%, and 100%. On the other hand, due to the high score for inter-observer agreement (85%) in the present study, bcl-2 staining may be considered as a diagnostic implement in equivocal cases of OKCs.

CONCLUSION

The expressions of bcl-2 demonstrated a frank difference in the biological character of OKCs, as compared to the other studied odontogenic

cysts. We suggest further investigation on the distribution of bcl-2 positive cells, either by evaluating primary and recurrent cysts or simple and syndromic OKCs.

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