

## RESEARCH ARTICLE

# Expression of Bcl-2 in Primary and Recurrent Odontogenic Keratocysts in Comparison with Other Odontogenic Lesions

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### Abstract

**Purpose:** To determine the biological behaviour of common odontogenic cystic lesions by analysing and comparing bcl-2 expression amongst them. **Materials and Methods:** Our study covered 90 formalin fixed paraffin embedded tissue samples: 26 primary cases each of radicular cysts (RC), dentigerous cysts (DC) and odontogenic keratocysts (OKC) and 12 of recurrent OKCs. Bcl-2 expression was analysed immunohistochemically and data analysis was accomplished using SPSS version 17.0. Means were taken for age while for gender and site of the lesions frequencies and percentages were determined. The Chi-square test was applied to evaluate any statistically significant difference of bcl-2 expression in these lesions and p value of  $\leq 0.05$  was taken as significant. **Results:** All the recurrent OKCs showed a strong positivity for bcl-2 that was absent in all of its primary cases (p value < 0.05). Although variation in expression of bcl-2 was not found to be statistically significant between RC and DC, however, it became significant when all primary cases of these common odontogenic lesions were compared. **Conclusions:** Recurrent OKC showed comparatively a more aggressive behaviour than their primary counterparts and also from RC and DC. Bcl-2 proved to be a valuable adjunct in determining aggressive biological behaviour of odontogenic lesions.

**Keywords:** Odontogenic keratocyst - radicular cyst - dentigerous cyst - Bcl-2 - KCOT

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### Introduction

Odontogenic cysts (OC) are one of the most frequently reported pathologies; characterized by pathological fluid filled cavities and lined by odontogenic epithelium. Radicular (RC), Dentigerous (DC) and Odontogenic Keratocysts (OKC) are reported the most commonly occurring amongst them (Naz et al., 2012). It is generally considered that degeneration or proliferation of epithelium of dental lamina or enamel organ may lead to their development (Singh and Gupta, 2010; Sujatha et al., 2013). These three lesions bear great interest because of their variable clinical behaviour. OKC however shows a different growth mechanism, a high recurrence rate (5-62%) and a biologically aggressive nature (Jahanshahi et al., 2006; Hyun et al., 2009). According to the most recent classification of 2005 is now considered as a benign cystic neoplasm known as Keratocystic Odontogenic Tumour (KCOT) (Philipsen, 2005). However, controversies still persist whether to consider it a cyst or a benign neoplasm (Li, 2011). The proliferative and apoptotic activity of its lining epithelium cells is believed to be one of the factors playing significant role in its growth and recurrence (Sujatha et al., 2013). Bcl-2 is an anti-apoptotic protein that prolongs the survival of cells by blocking apoptosis and promotes development of tumour (Jaafar et al.,

2012). Studies have been conducted in the past to detect its role in tumourigenesis, a few also to evaluate its role in determining the biological behaviour of odontogenic cysts (Zyada et al., 2009; Kelly and Strasser, 2011; Jaafar et al., 2012). This no doubt has done some part of work on the primary odontogenic cysts, still data regarding its expression in recurrent OC and the cysts showing dysplastic changes or malignant transformation of their epithelial lining is lacking. Moreover, results of various studies evaluating its role in odontogenic cysts have been conflicting that needs to be explored further. Keeping all this in view, the current study was planned to determine the biological behaviour of these common odontogenic lesions by evaluating the expression of bcl-2 immunohistochemical marker in them. The objective was to analyse expression of the bcl-2 oncoprotein in primary and recurrent OKC and its comparison with RC and DC in our set up.

### Materials and Methods

The paraffin embedded blocks of 90 selected cases of RC, DC and OKC diagnosed from January 2006 to December 2010 were retrieved from the archival records of Histopathology department of Armed Forces Institute of Pathology (AFIP), Rawalpindi (Pakistan). They

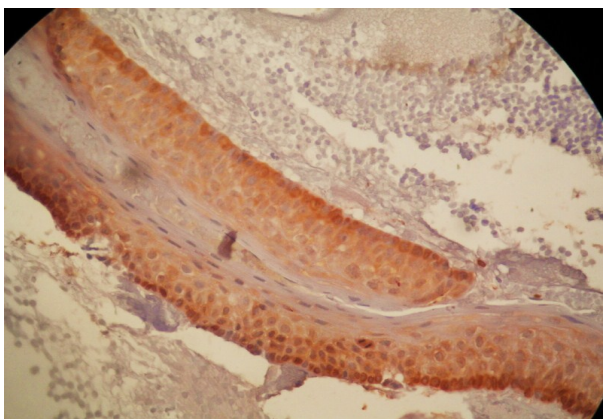
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consisted 26 primary cases each of RC, DC and OKC and 12 recurrent cases of OKC. All these samples were non syndromic and any having necrosed, scanty or autolysed tissue was excluded from study. The clinicopathological data of these cases was collected from the specimen's laboratory request forms and diagnosed on the basis of patient's history, radiographic findings and histological examination. To diagnose histologically, slides of each case were prepared by cutting fresh sections (3 to 5 µm) from paraffin blocks using Accu cut rotary microtome (SRM 200 Sakura, Japan). These were subsequently stained with H & E (Haematoxylin and Eosin) and applied with bcl-2 immunohistochemistry (Monoclonal antibody Bcl-2 of Vision biosystems Novocastra Kit).

For antigen retrieval, mounted sections on slides were deparaffinised, rehydrated (with distilled water) and to block any endogenous activity were placed for 10 minutes in 3% hydrogen peroxide. They were then microwaved four times for 5 minutes and incubated in tris buffered saline (TBS pH 7.6) for 5 minutes. To block any nonspecific binding were later diluted with normal horse serum for 10 minutes. These sections were incubated overnight at 4°C with primary antibody (bcl-2) and then washed in TBS (2-5 min). The secondary antibody was reacted for 60 min at room temperature (mouse EnVision System HRP, DakoCytomation). In order to visualize, slides were incubated in DAB (Diaminobenzidine) and then counterstained with haematoxylin, dehydrated and mounted.

The prepared slides were examined by a consultant histopathologist on Olympus BX60 microscope attached to a coloured video camera. Bcl-2 staining was evaluated in the epithelium and arbitrary counting of cells was done using 10 consecutive microscopic high power fields. Percentages of bcl-2 positive cells was calculated by dividing with total no of cells and categorized in 4 groups; 0-9% staining was considered as negative expression; 10-24% as mild or weak positive; 25-50% moderate positive and over 50% as strong positive. Tonsil tissue was taken as positive control and staining pattern was membrane and cytoplasmic.

For statistical analysis means were calculated for age while frequency and percentages were determined for gender and site respectively. Chi-Square test was applied

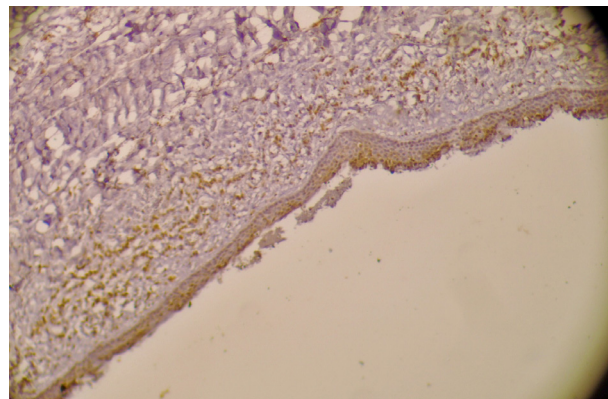


**Figure 1. Strong Positive Expression of bcl-2 Seen in Recurrent OKC**

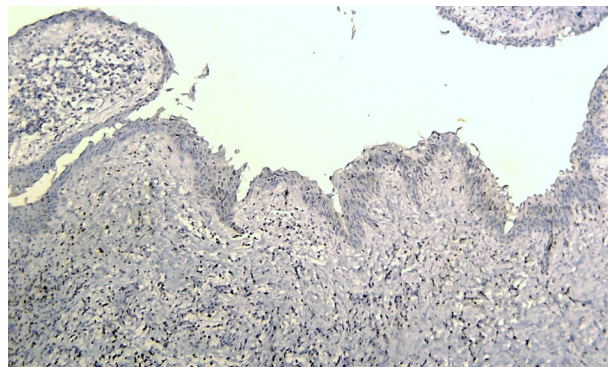
**Table 1. Expression of bcl-2 in primary OCs**

Bcl-2 Expression	Primary Odontogenic cysts			Total
	Radicular cyst	Dentigerous cyst	Odontogenic Keratocyst	
Negative	22	16	4	42
Mild/weak positive	4	6	6	16
Moderately positive	0	4	16	20
Strong positive	0	0	0	0
Total	26	26	26	78

p value<0.05



**Figure 2. Moderate Expression of bcl-2 Seen in Primary OKC**



**Figure 3. Negative Expression of bcl-2 Seen in Primary DC**

to evaluate any statistically significant expression of bcl-2 in these lesions. P value of ≤0.05 was considered significant and data was analysed using SPSS version 17.0.

## Results

We noticed that males were affected twice as compared to females (2:1) with mandible as the most prevalent site of involvement (64.4%) followed by maxilla (35.6%). The ages of patients ranged from 4 to 69 years having a mean of 28±17.23. The observed expression of bcl-2 in primary OCs is shown in Table 1; depicting its greatest expression in OKCs with none showing strong positivity for it. Comparison of expression of bcl-2 between RC and DC revealed statistically insignificant result (p value>0.05), which however became significant when the two were compared with primary OKC (p value<0.05). All 12 cases of recurrent OKC revealed a strong expression for

bcl-2 (Photomicrograph 1) that also proved statistically significant when compared with primary OKCs.

## Discussion

Bcl-2 has derived its name from B-cell Lymphoma-2; a second member of range of proteins initially described on chromosomes 14 and 18 in follicular lymphomas (Souers et al., 2013). It is a proto-oncogene that produces a protein found in endoplasmic reticulum, nuclear envelope and mitochondrial membrane. It is considered as anti-apoptotic protein (Serasinghe et al., 2014) which prolongs survival of cells by blocking apoptosis and promoting development of tumour (Zyada et al., 2009; Rooswinkel et al., 2014). So far limited work has been done on bcl-2 expression in OKC and other odontogenic cysts and most of it is confined to primary lesions. Likewise data revealing its expression in recurrent OKCs is also scarce.

Although variation in bcl-2 expression was seen in primary cases of these cysts but none revealed strong expression for it. A statistical significant difference was noted when all three were compared, which however was lacking between RC and DC suggesting their similar biological behaviour quite different from OKC. Our this finding is similar with an Iranian study that reported 19 of 20 OKCs were positive for bcl-2; just 1 case of both DC and RC showed positivity in the same number of cases. The comparison of three also revealed a statistically significant difference that remained insignificant between RC and DC (Jahanshahi et al., 2006). Another study by Italian group of researchers also revealed bcl-2 positivity in all 14 cases of OKCs and negativity in all 20 cases of RC; just 1 of the 19 DCs showed positivity for it. (Piatelli et al, 1998). These studies suggest a comparatively more aggressive biological behaviour of OKC than the other two. This might be the reason of rare recurrence and malignant transformation of RC and DC. A significant difference of expression of bcl-2 in primary and recurrent OKCs reflects a comparative less aggressive biological behaviour of primary OKC than their recurrent counterpart.

Kolar et al. (2006) analysed the expression of some apoptosis and proliferation markers in Nevroid Basal Cell Carcinoma Syndrome (NBCCS) associated OKC, sporadic OKC and other odontogenic cysts. They noticed a different immunophenotype pattern of dentigerous, radicular and non-specified odontogenic cysts from both NBCCS and sporadic keratocysts. The results in both types of OKCs were quite distinguishable from other odontogenic cysts that confirmed a different biological potential of all these odontogenic lesions. Similarly a Turkish study on evaluation of expression of immunohistochemical markers; bax, bcl-2, and Ki-67 in OKC in comparison with ameloblastomas and RC successfully established a significantly higher expression of bcl-2 in the whole thickness of OKC epithelium than RC. They suggested that odontogenic keratocysts bear a high proliferative and survival activity (Tekkesin et al., 2012). Likewise a study conducted in Israel showed significantly higher expression of bcl-2 in OKCs (primary OKCs and NBCCS-OKCs) than in DCs and RCs. They were of the view that odontogenic keratocyst most likely has a neoplastic nature

(Vered et al., 2009). A recent Indian study by Sujatha et al. (2013) also reported significant difference of bcl-2 expression in OKC, DC and RC which too supports our findings.

Contrarily, a Brazilian and Japanese study mentioned bcl-2 immunoreactivity in Radicular cyst as well (Loyola et al., 2005, Suzuki et al., 2005). Another Japanese study stated expression of bcl-2 protein in 23 of 41 primary OKCs and 9 of its 12 recurrent cases. They revealed that apoptotic factors in recurrent OKCs are not significantly different from primary OKCs (Kimi et al., 2000). These results no doubt are quite different from our study that might be resulted from difference in methodology or primary OKCs presented there comparatively had a more aggressive growth pattern.

We noticed expression of bcl-2 in the lining epithelium of basal and supra basal layers of odontogenic cysts with a strong expression in whole thickness of epithelial lining of recurrent OKCs. This demonstrates that bcl-2 inhibits apoptosis to promote cellular proliferation in the whole epithelial lining of OKC.

Recurrent OKC has a more aggressive biological behaviour than their primary counterpart and also from other odontogenic lesions. It has anti apoptotic activity in its epithelial lining which may be the cause of its aggressive behaviour and high recurrence rate. On this basis we can reinforce it as a tumour. Bcl-2 has proved a valuable adjunct in determining aggressive biological behaviour of odontogenic lesions. Therefore, follow up of odontogenic lesions bearing strong bcl-2 positivity is essential for a better prognosis. Further investigation on the role of bcl-2 in determining biological behaviour of these odontogenic lesions may be determined by evaluating its expression in their epithelial linings having dysplastic or malignant changes.

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