

## Distribution of Bcl-2 in Odontogenic Cyst and Tumours: Histochemical Study

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### ABSTRACT

**Introduction:** The Odontogenic Keratocyst is known for its aggressiveness, high recurrence rate and transformation of keratinized epithelia to non-keratinized squamous epithelium for which inflammation has been suggested to be responsible. bcl-2 an anti-apoptotic protein, prolongs the life span of epithelial cells and allows proliferation, differentiation and morphogenesis.

**Materials and method:** Study was carried out comprising of 90 cases; [30 Ameloblastoma, 30 Keratocystic Odontogenic tumor and 30 Radicular cyst]. Bcl-2 expression was determined with respect to Localization, Area [percentage] and Intensity of stained cells in epithelium and connective tissue stroma by counting the endothelial, round and fusiform cells.

**Results:** In epithelium bcl-2 expression in Keratocystic Odontogenic tumors was higher followed by Ameloblastoma and lowest in Radicular cyst. Whereas, in connective tissue stroma bcl-2 expression was higher in Keratocystic Odontogenic tumor and Radicular cyst than Ameloblastoma cases. Solid variants showed statistically higher expression as compared to the Unicystic variants of Ameloblastoma [p-value 0.009, 0.033, 0.011, 0.041].

**Conclusion:** High expression of bcl-2 in KCOT supports the general agreement that some features of OKC are those of a neoplasia. The bcl-2 expression in connective tissue cells suggests that these cells may also be important as epithelial cells in the biological behaviour odontogenic keatocyst.

**Keywords:** Ameloblastoma, apoptosis, bcl-2, keratocystic odontogenic tumor, radicular cyst

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## INTRODUCTION

A dysfunctional apoptotic system can lead to either excessive removal or prolonged survival of cells. Therefore, Dysregulation of apoptosis is involved in the pathogenesis of a variety of diseases such as cancers, viral infections and immunological disorders (1).

The center-piece of the apoptotic program and the major effector arm of the cell death program is the bcl-2 family of related proteins. The prototype member of this family is bcl-2, an acronym for B-cell lymphoma/leukemia-2 gene, that was first discovered at the breakpoint of the [14;18] in a follicular non-Hodgkin's B-cell lymphoma. The proteins encoded by the bcl-2 family localize to the outer mitochondrial membrane, the nuclear membrane, and the endoplasmic reticulum (2).

Immunoreactivity for bcl-2 protein has been detected in enamel organs and dental lamina of tooth germs as well as in odontogenic lesions like Ameloblastoma, Keratocystic Odontogenic tumor, radicular cyst etc. to elucidate any relationship between histological features and biological potential (3).

Ameloblastoma is the most most significant odontogenic neoplasm of concern.<sup>[5]</sup> Several recent studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors however; the detailed mechanisms of oncogenesis, cyto-differentiation, and tumor progression remain unknown (4).

Odontogenic Keratocyst, another common cyst arising from dental lamina or remnants of the dental lamina, has been deeply studied due to its aggressive clinical behavior with high recurrence rates, distinct histopathologic features, singular growth mechanism, and genetic alterations.(5) The potential for aggressive clinical behaviour and local recurrence resulted in its recent classification as a benign odontogenic tumor with a new nomenclature: keratocystic

odontogenic tumor (6). Several studies have demonstrated the higher proliferation activity of the epithelial lining in keratocystic odontogenic tumors [KCOTs] in relation to odontogenic cysts. However, despite the fact that cell turnover is controlled by both cell proliferation and apoptosis, few studies have evaluated apoptosis related proteins and apoptotic index in the epithelial lining of the odontogenic cysts (7).

Generally, most of the Immunohistochemical studies on bcl-2 expression included epithelial cell examination. There are a few studies in the literature examining bcl-2 expressions in odontogenic epithelium and connective tissue cells of oral lesions. Hence, the present study was aimed to evaluate bcl-2 expression and its distribution in the epithelial lining as well as connective tissue cells of Ameloblastoma, Keratocystic Odontogenic tumor and Radicular cyst.

## **MATERIALS AND METHODS**

**Sample selection:** The present study was carried out on 120 formalin-fixed paraffin-embedded tissues 40 each of Ameloblastoma, Keratocystic Odontogenic tumor and Radicular cyst samples, which were retrieved from the archives of Department of Oral and Maxillofacial Pathology. Relevant clinical details were obtained as per the case history Performa. All the other relevant patient clinical data was also tabulated. None of the KCOTs were associated with Nevroid basal cell carcinoma syndrome [NBCS].

**Evaluation methods:** Stained sections were examined in Olympus BX60 microscope attached with a colour video camera [Olympus Analysis Five]. All stained areas demonstrating positivity for bcl-2 were determined with respect to Localization, Area [percentage] and Intensity of stained cells at a magnification of  $\times 40$  and the number of positively stained cells was counted in

10 representative areas of the epithelium using a  $\times 40$  objective in a minimum of 100 cells in the full length of the epithelium. The expression of bcl-2 was also determined in the connective tissue stroma, by counting the endothelial, round and fusiform cells at a magnification of  $\times 40$  and the number of positively stained cells was counted on 10 representative areas as specified by Tenkkesin MS et al 2012.

The Area of staining [Immunoreactivity] in the epithelium was defined by: [Vered M et al, 2009]: [0] no staining; [1] low staining 1% to 10% positive; [2] intermediate staining 11% to 50% positive; [3] high staining more than 50% positive. Whereas, the intensity of bcl-2 positivity was estimated as follows [Jahanshahi GH et al, 2006]: [-] less than 5% stained cells, [ $\pm$ ] 5-9% cells stained positively, [+] between 10 and 24% cells stained positively, [++] between 25 and 50% cells stained positively and [+++] Greater than 50% of tumor cells stained positively. 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,  $\times 100$ ] and also approximal to bcl-2 negative cases [Jahanshahi GH et al, 2006]. 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 [+++].

The number of positive cells was divided into the total number of cells counted in the whole area. The result was multiplied by 100 to find the percentage of positive cells. Collected data was analysed using the SPSS software for Windows [Version 17]. One way ANOVA [F-test] was carried out for comparing the parameter for multiple groups. Comparison between groups was carried out using the Student's 't' test. Correlations between bcl-2 positivity in different lesions and degree of inflammation were derived using Karl Pearson's, with the level of significance set at  $p \leq 0.05$ .

## RESULTS

Positive bcl-2 expression was considered in the epithelium of the odontogenic lesions when at least 10% of the cells were stained. All Keratocystic odontogenic tumors [Fig. 1a] and Ameloblastoma [Fig. 1b] and 10 out of 40 radicular cysts [Fig. 1c] were positive for bcl-2.

In 26 of the 38 stained KCOTs, positively stained cells were observed in the basal layer while in the other bcl-2 positive KCOTs [14 of 40], the stained cells were in the basal/supra basal region. In radicular cyst positive cells were located in the basal/supra basal layers of only 4 cases. In Ameloblastomas bcl-2 was detected mainly in the peripheral layer whereas only a few cells were positively stained in the central layer of epithelial tumor islands [8 cases].

Higher bcl-2 staining area and intensity of the connective tissue was found in keratocystic odontogenic tumor as compared to radicular cysts and least in ameloblastomas. All Keratocystic odontogenic tumors [Fig. 1d], 38 out of 40 Ameloblastoma [Fig. 1e] and 38 out of 40 radicular cysts [Fig. 1f] were positive for bcl-2.

Mean and standard deviation values of bcl-2 staining area and intensity of the stained cells in the epithelium and connective tissue of studied odontogenic lesions is shown in Table I. Significant difference were observed between Ameloblastoma, KCOT and Radicular cyst. In multiple comparisons, bcl-2 staining area and intensity revealed a statistically significant difference between KCOT and Ameloblastoma [Table 2] and between Ameloblastoma and Radicular cyst [Table 3]. There was no significant difference of bcl-2 staining area and intensity between Radicular cyst and KCOT in the connective tissue, whereas epithelial bcl-2 staining area and intensity were significantly higher in Keratocystic Odontogenic tumors.

In both variants of ameloblastomas Solid Ameloblastomas lesions had a statistically significant high score of bcl-2 staining area and intensity both in the epithelium and connective tissue [Table 5, Fig 2 a & b]

Radicular cyst displayed highest degree of inflammation, Keratocystic odontogenic tumor also displayed a considerable inflammatory component, whereas Ameloblastoma demonstrated a mild to intermediate degree of chronic inflammation.

## **DISCUSSION**

In the present study, odontogenic epithelium of Keratocystic Odontogenic tumor revealed strong positive staining for bcl-2 in the whole thickness of epithelium, in basal and supra basal layers and low expression of bcl-2 in the basal and suprabasal layers in Radicular cysts cases. These findings are in accordance with earlier studies done by Piatteli A et al 1998(8) and Jahanshahi Gh. et al in 2006(9).

Ameloblastoma in the present study revealed medium expression of bcl-2 in the peripheral cell layer of the tumor islands and low expression in the central stellate reticulum like layer. These findings are in accordance with earlier studies done by Mitsuyasu et al 1997(10) and Sandra et al 2001.(11) In a study by Florescu A et al in 2012,(12) bcl-2 expression was present in 88.23% of the investigated ameloblastomas, predominantly in columnar cells from the peripheral zone. Similar studies in the literature communicate that around 90% of ameloblastomas are positive for bcl-2 similar to our study, which indicates that bcl-2 expression may be related to differentiation and proliferation of odontogenic epithelium, and bcl-2 overexpression may be associated with the ameloblastoma development.(13,14,15) These results

indicate that in ameloblastomas bcl-2 protein could function primarily as anti-apoptotic factor, which reflects the proliferative activity of neoplasms. Also, the expression of bcl-2 suggests the aggressive nature of odontogenic tumor and these results will be beneficial in the differential diagnosis of odontogenic tumors and other tumors that occur in the mouth. In addition, it is estimated that the bcl-2 protein may play a role in maintaining stem cell population in peripheral layers of tumor islands of which are recruited proliferating cells.

In the connective tissue stroma bcl-2 area and intensity in Keratocystic Odontogenic tumor and Radicular cyst was higher than Ameloblastoma. Low positivity in Ameloblastoma may be attributed to the mature fibrous connective tissue stroma as compared to the highly inflamed stroma of the odontogenic cysts. These results are in contrast to study done by Tekkesin MS et al in 2012(7) in which there was no significant difference in expression of bcl-2 between Keratocystic Odontogenic tumor and Ameloblastoma, as well as between Radicular cyst and Ameloblastoma. So far in the literature no studies have been cited that have compared the staining intensity of bcl-2 in these 3 lesions only the area of bcl-2 staining has been compared.

In the present study, overall comparison for the expression of bcl-2 in all 3 odontogenic lesions revealed statistically significant higher expression in the whole thickness of epithelium of Keratocystic odontogenic tumor and predominantly in the peripheral cell layer of the tumor islands of 8 ameloblastomas. On the other hand, Radicular cyst samples revealed very low bcl-2 expression. These results are in accordance with that found by Mitsuyasu et al in 1997(10) and Sandra et al in 2001.(11) This could lead to aggressive growth pattern of Keratocystic Odontogenic tumor and Ameloblastoma.

In the present study inter-comparison between different variants of Ameloblastoma revealed a significantly higher expression of bcl-2 area and intensity both in the epithelium and

the connective tissue of Solid or Multicystic variants as compared to the Unicystic variants of Ameloblastoma. These results are in accordance with the study done by Vered M et al in 2009(18) where SAM demonstrated intermediate and low scores in basal and stellate reticulum layers and only a few SAM samples had a high score similar to our study, on the other hand UAM samples demonstrated less positivity for bcl-2 expression and one case of UAM was negative for bcl-2.

Study by Vered M et al. 2009, (18) provide further support to the assumption that an OKC can be of a neoplastic rather than a cystic nature. This is based not only on the expression of the PTCH and its downstream factors, SMO and GLI-1, but also on the analysis of the immunohistochemical profile of OKC, which is comprised of the SHH-related proteins and the SHH-induced bcl-2 oncoprotein. The quality and quantity of the interactions between the SHH and other cell cycle regulatory pathways most probably work synergistically to define the individual phenotype and the corresponding biological behaviour of OKCs. The activation of atleast two cell cycle regulatory systems is 'switched on' in OKCs as this lesion evolves. Investigations on the Immunoreactivity of bcl-2 protein have been demonstrated in KCOTs and recent studies report that bcl-2 positive cells are predominantly located basally, thus supporting the concept that apoptosis does not occur in the basal cells of the lining epithelium. However, TUNEL-positive cells have been detected exclusively in the surface layer of KCOTs, indicating marked levels of apoptosis. Thus, bcl-2 inhibits apoptosis to facilitate cellular proliferation in the basal and suprabasal layers, whereas apoptosis maintains the homeostasis of the thickness of the lining epithelium and allows the synthesis of large amounts of keratin in the surface layer of KCOTs. Considering that there is a regulated balance between cell proliferation, cell differentiation and cell death in this type of lesion, this may explain why KCOTs, though



portraying a neoplastic behaviour, with an increase potential to proliferate, do not tend to form tumor masses (19).

## **CONCLUSION**

In conclusion, the results of the present study suggest that Odontogenic Keratocyst has a high proliferative and survival activity and that might be one of the reasons why Odontogenic Keratocyst has a high recurrence rate. The proliferation potential of the epithelium and the overexpression of various anti-apoptotic proteins in odontogenic epithelial tumors are quite significant for their clinical behaviour providing further support to the assumption that an OKC can be of a neoplastic rather than a cystic nature. We have also demonstrated that connective tissue cells may also be important as epithelial cells in the biological behaviour of these odontogenic lesions. Although these results support that Keratocyst Odontogenic tumor is a neoplasm, with expression of bcl-2 similar to Ameloblastoma rather than Radicular cyst, there are not enough genetic studies and only two studies so far have directly compared the expression of bcl-2 in these three lesions all together. Further studies on genomic changes may help better understanding of the pathogenesis of odontogenic keratocysts.

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Table 1: Overall mean and standard deviation values

<b>Odontogenic lesion</b>	<b>Area</b>		<b>Intensity</b>	
	Epithelium	Connective Tissue	Epithelium	Connective Tissue
<b>Ameloblastoma</b>	28.64± 22.381	16.575± 11.135	23.405± 24.359	13.420± 12.094
<b>Keratocystic Odontogenic tumor</b>	49.605± 24.786	45.045± 27.176	44.510± 26.126	43.815± 28.709
<b>Radicular cyst</b>	9.680± 6.951	38.600± 23.385	11.065± 14.124	38.060± 24.158
<b>“p-value”</b>	<b>0.00 [S]</b>	<b>0.00 [S]</b>	<b>0.00 [S]</b>	<b>0.00 [S]</b>

Bcl-2 in Odontogenic Cyst and Tumour

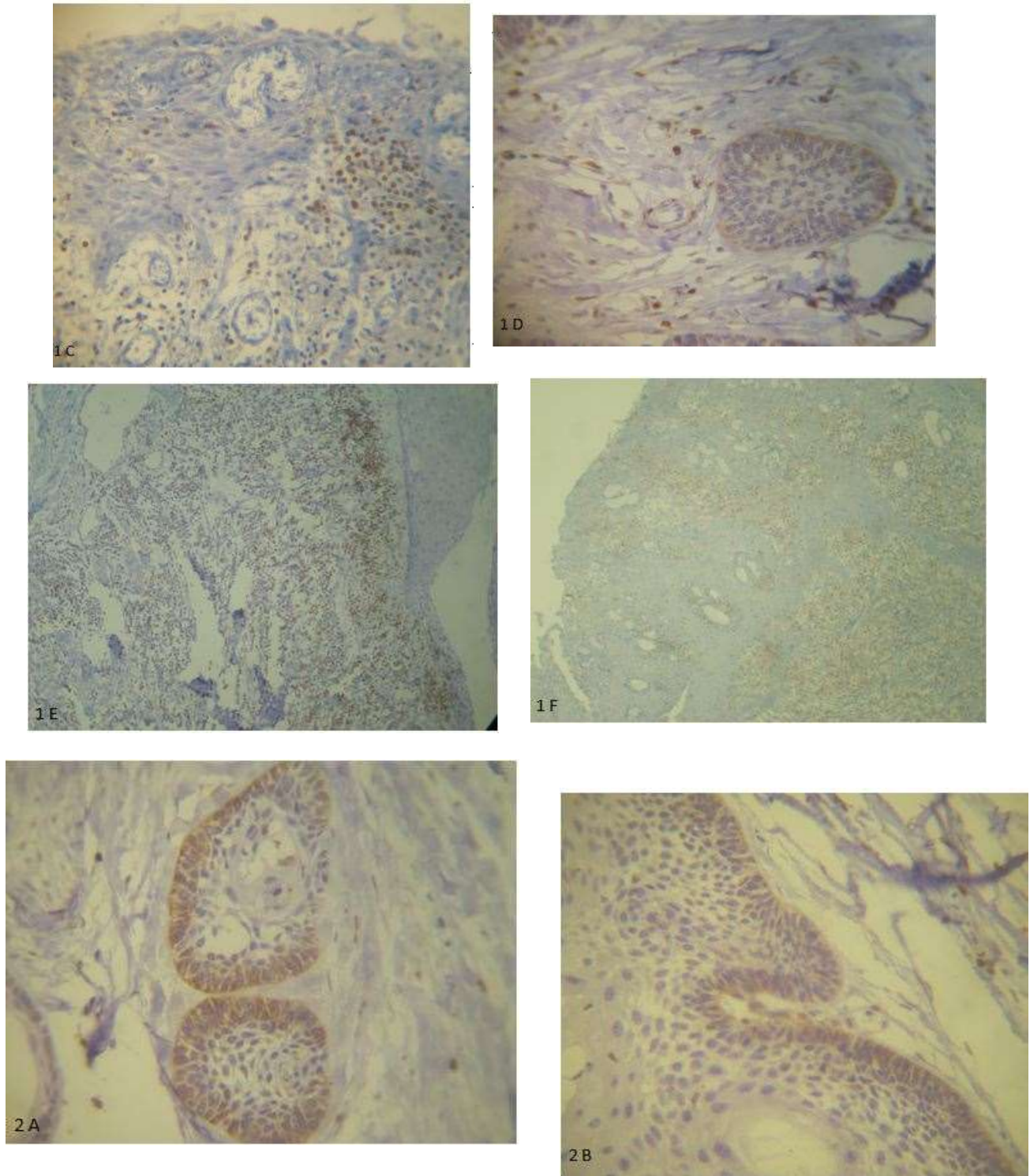


Fig. 1: bcl-2 expression in the epithelium of a) Ameloblastoma, b) KCOT, c) Radicular cyst and bcl-2 expression in the connective tissue of d) Ameloblastoma, e) KCOT, f) Radicular cyst respectively



Fig. 2: bcl-2 expression in the variants of Ameloblastoma a] solid or multicystic, b] Unicystic respectively