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RESEARCH ARTICLE

IMMUNOHISTOCHEMISTRY WITH BCL2 (ANTIAPOPTOTICMARKER)-IN SURGICAL ENDODONTICS

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ABSTRACT

Aim: To analyze bcl-2 protein as marker of regulating apoptosis in periapical tissue reaction which causes bony defects
Objective: Immunohistochemical study of periapical lesion gives the clinician a chance to correlate between case history findings and progression of the lesion.
Materials and method: Six patients with the history of trauma and having large periapical radiolucency, indicated for periapical curretage were selected. The periapical curretted biopsy specimens were formaline fixed, taken for histopathological and immuno histochemical study. The bcl2 expressions in all specimens were studied.
Inference: Bcl2 proteins were positive in all cases, but more in cystic lesions
Conclusion: bcl2 proteins were used as markers to understand the extent of the lesion. More bcl2 expressions shows blocked apoptosis, which leads to bony lesions.

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INTRODUCTION

The immunopathological activity taking place in the development of periapical lesions need to be ascertained. Because in majority of cases, symptomatic as well as asymptomatic cases responds differently to the pathological processes involved in the lesions and its sequelae. Hence, the rationale behind them need to be understood. The addition of methods like detection of immunohistochemical markers like bcl2 and caspases helps us to understand the pathological process, their aggressiveness, helps us to evaluate the therapeutic means with their prognosis. This case series study gives insight as well, help us to understand the intricacies involved in the bone destructions caused by periapical lesions.

BCL2 FAMILY

Apoptosis is the programmed cell death in which cells activate enzymes that degrade nuclear DNA and protein. It plays various roles in the organization of normal tissues and their pathogenesis by modulation of several proteins. The bcl-2 family is a group of closely related proteins that plays a major role in apoptosis regulation.

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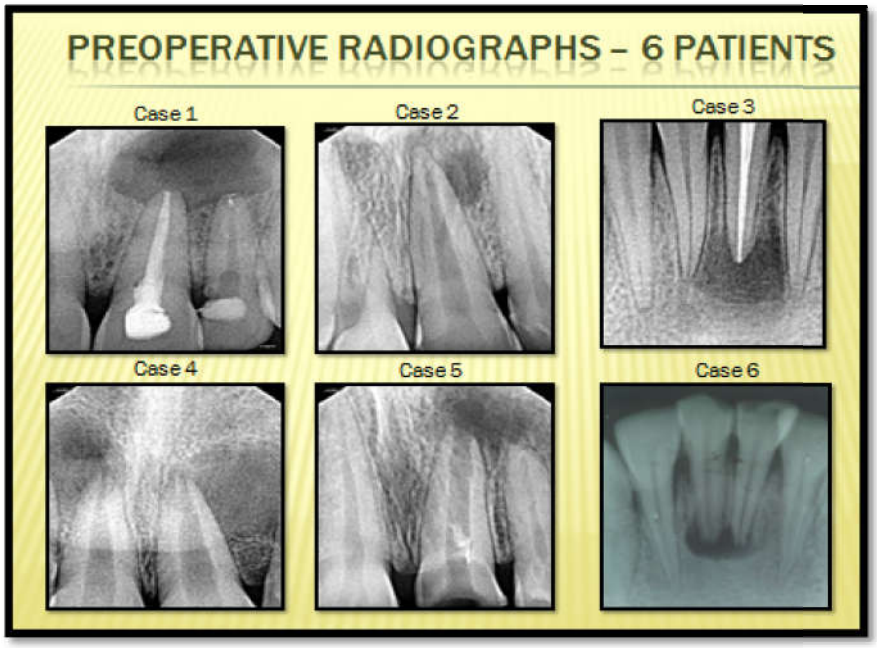
This is constituted by inductive (e.g., bax) and inhibitory (e.g., bcl-2) apoptotic factors, and cell survival is warranted by higher inhibitory apoptotic gene expression. The bcl-2 protein is a 26-kda putative member which acts as a cell death suppressor thus facilitating cell survival by regulating apoptosis. Accumulation of cells with an aberrant bcl- 2 expression could be an important step in pathogenesis. Bcl-2 expression could probably be related to loss of cell differentiation. Other proliferative apoptotic markers are p53, ki67 (Sujatha *et al*., 2013). Immuno-reactivity for Bcl-2 family proteins has been detected in Odontogenic epithelium under various conditions, suggesting that these proteins play a role in regulating Cellular kinetics of odontogenic epithelium. The aim of this present study was to analyze bcl-2 protein as marker of regulating apoptosis in periapical tissue reaction which causes bony defects MATERIALS AND METHODS:
Six patients who were reported to the department of conservative dentistry and endodontics, Best dental science college, Madurai, presenting with history of trauma more than five years and having periapical radiolucency of the involved teeth were selected. Their clinical and radiological features (fig1) were given in Table 1. These patients were indicated for surgery andperiapical surgery was done in the department. Periapicalcurretted biopsies were stored in 10% formalin and taken for histopathological study and immunohistochemical analysis.

Out of six specimens, along with the clinical feature and histopathologicalstudies (Fig 2, Table 2,3), case 1, 4 and 5 showed cystic features. The case 5 and 6 were periapical granuloma. The case 2 showed chronic inflammatory condition.

Observations

In our study Bcl2 staining is positive in all the cases.The intensity of Bcl2 positive is estimated as[(-)fewer than 5% positive cells or no staining, (+) 5-9% positive, (++) 10-25%



CASE NO	1	2	3	4	5	6
TOOTH	21,22	11,21	31	11,21	21	31,41
PERIODONTAL SPACE WIDENING	✓	✓	✓	✓	✓	✓
PERIAPICAL RADIOLUCENCY	✓	✓	✓	✓	✓	✓
MARGINS	LARGE CIRCUMSCRIBED SCLEROTIC MARGIN	DIFFUSED ILL DEFINED	CIRCUMSCRIBED	CIRCUMSCRIBED SCLEROTIC	DIFFUSED ILL DEFINED	WELL DEFINED CICUMSCRIBED
OPEN APEX					✓	✓
CRESTAL BONE LOSS						✓
ROOT RESORPTION	✓				✓	
OTHER	RADIO OPACITY IN ROOT CANALS, CERVICAL RESORPTION					

Table 1. Radiographic features of 6 cases

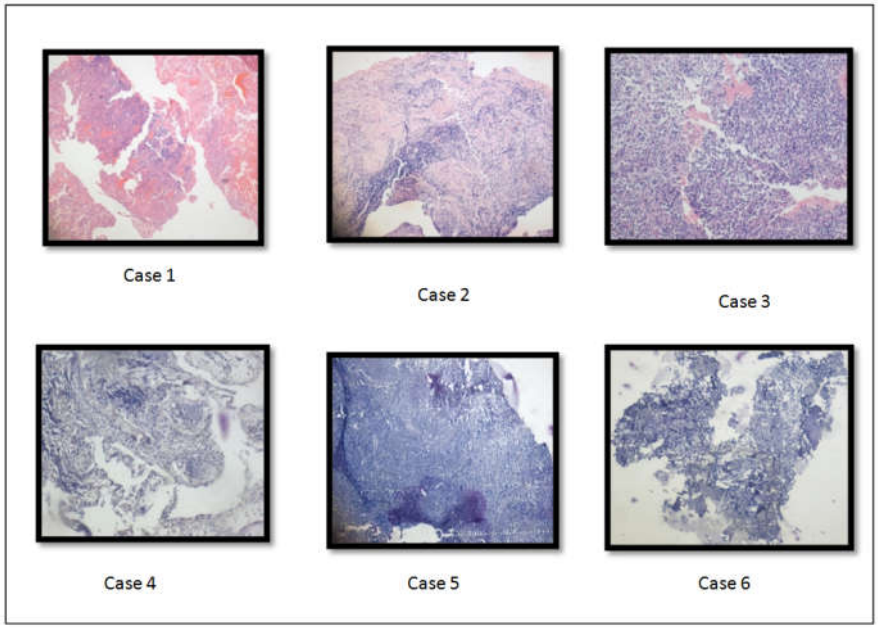
While preparing the sections, successional sections have to be taken for histopathological and immunohistochemical study. They were Polylycin coated, over night incubated and deparaffinized. The xylene buffer is added to it, heated in microwave and then cooled.

The 3% hydrogen peroxide is added for 5 mins. The primary and secondary antibody added(poly xl binder 10mns, poly HBR 10mns)at room temperature. The Buffer wash Diaminobenzoidine- conjugate is added. The Haematoxylin counter staining was done. They were rehydrated and mounted. The Positive bcl-2 expressions were seen as light brown, granular stain in cytoplasm of cell.

positive, (+++) 25-50% positive, (++++) more than 50% positive[2].Comparatively the staining is more in cystic lesions(fig 4) case 1, case 4, i.e staining is positive in epithelial cells, than granulomas(fig 3) case 5,case 6 where only lyphoid cells positive. Bcl-2 staining in various periapical lesions gives the understanding about the extent of apoptosis occured in those lesions.

DISCUSSION

Although many professionals prefer the surgical removal of lesions which is seen radiographically, compatible with radicular cysts, studies have shown high rates of repair of these lesions by endodontic therapy.



HISTOPATHOLOGICAL REPORT

MICROSCOPIC FEATURES

AGE/SEX	28/M	32/M	17/M	17/M	18/M	23/M
TOOTH NO	21 22	11 21	31 41			31 41
PROVISIONAL DIAGNOSIS	PERIAPICAL LESION	PERIAPICAL LESION	PERIAPICAL LESION	PERIAPICAL LESION	PERIAPICAL LESION	PERIAPICAL LESION
COLOUR						
GW	v	v v	v v	v	v	v v
GB	v					
MICROSCOPIC FEATURE						
LINING EPITHELIUM	v	v	v	v		
FIBROUS CONNECTIVE TISSUE		v		v	v	v
NON KERATINISED	v	v	v	v	v	v
SQUAMOUS EPITHELIUM	v			v		
ARCADING				v		
INTERVENING FIBROUS CONNECTIVE TISSUE				v		
DENSE COLLAGEN FIBERS		v		v	v	LOOSE COLLAGEN FIBERS v
THICK BUNDLES					v	
PARALLEL TO LINING EPITHELIUM				v		
CHRONIC INFLAMMAORY FEATURE		v	v	v		v
DIFFUSE				v	v	v
LYMPHOCYTES		v	v			v
PLASMA CELLS						
FEW			v	v	v	v
INCREASED	v					
IMPRESSION	RADICULAR CYST	INFLAMMATORY CYSTIC LESION/ CHRONIC NON SPECIFIC INFLAMMATION	INFECTED APICAL CYST/ CHRONIC ABSCESS	PERIAPICAL CYST	PERIAPICAL GRANULOMA	PERIAPICAL GRANULOMA
IMMUNO HISTOCHEMICAL STUDY						
BCL2	v	v	v	v	v	v

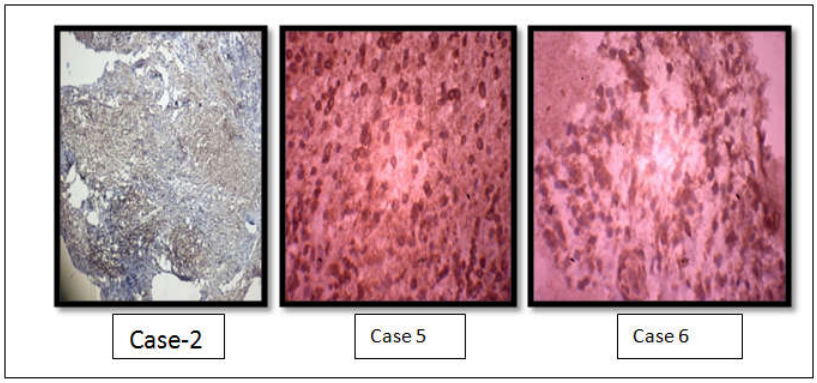


Fig.3. Case-2 Chronic nonspecific inflammation,Bcl-2 positive(2+) inlymphoid cells. Case 5, Case 6 – Periapical granuloma –Bcl-2 positive(2+) in lymphocytes.

Fig.4 Case1,3,4- Periapical cyst mostly positive in epithelial cells positive(2+)

Biologically, there are many hypotheses to explain the mechanisms of repair or regression of cysts after endodontic therapy. The epithelial cells Of the cyst lining may stop proliferating because of reduction in inflammatory mediators, growth factors, and cytokines. The lack of favorable environmental factors in the tissue and the presence of apoptosis prevent cell proliferation and consequently, the growth of the lesion. Apoptosis is a single deletion of scattered cells by fragmentation into membrane-bound particles that are phagocytised by other cell. It is essentially called cell suicide (Kerr, 1972). At appropriate time, under certain conditions, cells self-destruct without damaging adjacent cells. This process occurs constantly and can happen to seemingly healthy cells.

The entire process of apoptosis takes about 1 h from initiation. The major stages of apoptosis include the initiating trigger which leads to activation of intracellular mechanism for apoptotic process, Morphological changes of cell include cell membrane changes that signal phagocytic cell recognition and elimination without promoting inflammation. The initiating triggers are many, varied, and grouped broadly as physiological or non-physiological. Some are listed here Fas ligands (Fasl),tumour necrosis factor (TNF), nerve growth factor (NGF), nitricoxide (NO), lipopolysaccharide(LPS), host immune reactions, kinins and glucocorticoids (McKenna *et al*.1998). Two main cell death mechanisms have been described: necrosis and apoptosis (Searle, 1982; Blagosklonny, 2003; Loro, 2003; Satchell *et al*., 2003; Loreto *et al*., 2013). Necrosis involves massive tissue destruction induced by environmental signals that exceed cell adaptive responses. Apoptosis is a genetically programmed mechanism with a large role in cell population homoeostasis (Blagosklonny, 2003; Satchell, 2003). It can be triggered by the intrinsic, mitochondria-mediated pathway or by the extrinsic pathway, induced by death signalling ligands like TNFa and FasL (Charriaut-Marlangue & Ben-Ari1995, Adams & Cory 1998, Green & Reed 1998, Ferri & Kroemer 2001). The ultimate executioners of the apoptotic cascade are caspases, enzymes that cleave proteins resulting in cell destruction (Krammer1999, Scaffidi *et al*. 1999). Some of these proteins act as inducers/initiators (caspases-2, 8 and 9) or as executioners(caspases-3, 6 and 7) (Park *et al*. 2005) Caspase-3 is widely held to be the hallmark of apoptosis, the point of no return for cell death(Gr€utter 2000, Gamonal *et al*. 2001, Lawen 2003, Doonan & Cotter 2008).

Tekkesin *et al*. (2012) noted that bax- a pro-apoptotic molecule of intrinsic pathway, is overexpressed in epithelial and connective compartments of radicular cyst compared with keratocysts/ameloblastomas and that bcl-2 (an anti-apoptotic molecule), ki-67 (a cell proliferation marker) showed an aggressive behaviour. Growth factors and other survival signals stimulate the production Of bcl-2 (antiapoptotic protein), which is located in the mitochondrial membrane and in the cytoplasm and acts as a cell death suppressor and promotes cell survival (Caroline Alberici Martin, 2001 ; Louis *et al*., 2007; Nair *et al*., 1999).

Chronic Inflammation: It includes mononuclear cell infiltration leading to tissue destruction or necrosis. As a result, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of inflammatory granulation tissue (Nair *et al*., 1999; Takahashi, 1998).

Ystic Changes: The initial reaction leading to cyst formation is a proliferation of the epithelial rests in the periapical area involved by the granuloma. The activated T cells in the periapical granulomas produce lymphokines that may act on the rest of malassez causing proliferation and altered differentiation, leading to cyst formation (Takahashi, 1998; Tzifi *et al*., 2011; Suzuki *et al*., 2005).

Inference bcl-2 staining

Bcl-2 positivity could point to an abnormal control of the cell cycle. Its abnormal expression is an indicator of blocked apoptosis in periapical region, used as marker in identification and detection of cysts. More the inflammatory condition, less the apoptosis and more the staining, which helps to understand the factors related to cell death in epithelial lining of cysts. All the six cases revealed-increase in bcl-2, indicating disturbed apoptosis, and thus ideal treatment option of surgical endodontics is understood. Patients with minimal radiolucent lesion, history of acute inflammation, immunohistochemistry would reveal absence of bcl-2 (Fernandes, 2010), more suggestive of non surgical endodontic management. During wound healing, majority of inflammatory cells are no longer needed and are deleted by apoptosis. If apoptosis fails to occur, inflammatory reaction will continue to persist because of release of proinflammatory intracellular contents into the surrounding tissues.

The occurence of apoptosis helps in wound healing, Similar studies were done. Piattelli in 1998 studiedImmunoreactivity of bcl2 in odontogenic keratocyst (OKC)which showed strong positivity in basal cell layer. In dentigerous cyst (DC) only few positive cells in basal, para basal layer was seen. Ghjahanshahi in 2006 studied Bcl2 staining which showed significant difference OKC >RC(Radicular cyst),OKC>DC. Edamatsu (Edamatsu *et al*., 2005) studied FAS, bcl 2, Ki 67, ssDNA in dental follicles DC, for possible role of apoptosis in pathogenesis of DC in follicles. Bcl 2 was weak in epithelial cells neighbouring the basement membrane and their expression was lower in follicles than cysts. Eisuke kichi (Kichi *et al*., 2005) studied Cell proliferation, cell death, and expression of apoptosis- related proteins in the lining cells of OKCs, DCs, bcl2 positive ratio in entire layer of lining epithelium .it was 32% in OKC (Kichi, 2005). Caroline Alberici martin et.al studied Immunoreactivity of bcl 2 in17 RC and 9 DC Bcl 2 significantly higher in basal layer of DC than RC. R.Sujatha et.al studied Bcl-2 protein expression in 15 OKC, 15 DC, 15 RC. DC- 2 cases showed 25-50%, 3 cases showed 10-24% ,RC-9 cases showed less than 5% of cells

Conclusion

Immunohistochemical studies play a vital role in understanding periapical pathological process. Bony defects are caused by inflammatory process, anti apoptosis and cystic changes This study enables understanding of apoptotic reaction as an innate process which can be correlated from history of the patient

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