Original Article

Immunohistochemical analysis of factors related to apoptosis and cellular proliferation in relation to inflammation in dentigerous and odontogenic keratocyst

Gadiputi Sreedhar, M. Vijaya Raju[1], Kiran Kumar Metta[2], SM Manjunath[3], Sujan Shetty[4], Rakesh Kumar Agarwal[5]

Department of Oral Pathology, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh, [1]Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Amargadh, Bhavnagar, Gujarat, [2]Department of Conservative Dentistry and Endodontics, MIDSR Dental College and Hospital, Latur, Maharashtra, [3]Department of Oral Pathology, MM College of Dental Sciences and Research, Mullana, Amabala, Haryana, [4]Department of Periodontics, RKDF Dental College and Research Centre, Bhopal, [5]Department of Pedodontics, KMCT Dental College Kozhikode, Kerala, India.

Address for correspondence:
Dr. Gadiputi Sreedhar, Department of Oral Pathology, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh.
E mail: gadiputisreedhar@gmail.com

Abstract

Background: The effect of inflammation on pathogenesis and biological behavior of odontogenic keratocyst (OKC) and dentigerous cyst (DC) is not completely understood. Hence, we aimed to analyze the effect of inflammation on biological behavior of OKC and DC using a proliferative and anti-apoptotic marker, i.e., proliferative cellular nuclear antigen (PCNA) and Bcl-2, respectively. **Materials and Methods:** Immunohistochemical staining was performed using anti-PCNA and Bcl-2 antibody in 10 cases each of classical OKC, inflamed OKC and classical DC and inflamed DC. **Results:** Inflamed OKC and DC showed a significant increase in PCNA expression and decrease in Bcl-2 expression when compared with non-inflamed cyst. Correlation between inflammation and proliferative and anti-apoptotic activity was found to be statistically non-significant. **Conclusion:** Inflammation is responsible for change in behavior of neoplastic epithelium of OKC and hence should be treated meticulously, whereas in DC it is responsible for changes in the epithelial lining.

Key words: Apoptosis, dentigerous cyst, odontogenic cyst, odontogenic keratocyst, proliferative cellular nuclear antigen

INTRODUCTION

Odontogenic keratocyst (OKC) is a clinicopathologically distinct form of odontogenic cyst known for its aggressiveness and high recurrence. World Health Organization (WHO) has recently recommended the term keratocystic odontogenic tumor considering its neoplastic nature.[1] Under the influence inflammation OKC has a tendency to transform the keratinized epithelial lining to non-keratinized, whereas inflamed dentigerous cyst (DC) is seen to be lined chiefly or entirely by non-keratinized stratified squamous epithelium of variable thickness, sometimes with anastomosing rete ridges.[2,3] Nonetheless, the effect of inflammation on the epithelium of odontogenic cysts remains a subject of controversy, with contradictory results being portrayed.[3,4] Hence, the present study is aimed to evaluate the influence of inflammation on immunohistochemical (IHC) expression of proliferative cellular nuclear antigen (PCNA) and Bcl-2 in order to determine its bearing on behavior of odontogenic cysts.

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MATERIALS AND METHODS

It was a retrospective study approved by the institution ethics committee. A total of 40 formalin-fixed

paraffin-embedded tissue specimens of histopathologically confirmed cases of 20 parakeratotic type OKC (10 inflamed and 10 classical) and 20 DC (10 inflamed and 10 classical) were taken and diagnoses were made on the histopathologic criteria given by WHO in 1992.[5] 4 μm thick sections were taken and stained for PCNA and Bcl-2 using IHC method. The inflammatory density score was determined by counting inflammatory cells adjacent to the basement membrane, to a depth corresponding with one histopathological field (HPF) fewer than 400 magnifications. It was recorded in 10 separate fields and inflammatory density score for each case was calculated as the average of all HPFs examined. Inflammatory density was graded on a 4-grade scale. Grade 0: No inflammation, Grade 1: <15 cells/field, Grade 2: 15-50 cells/field, Grade 3: >50 cells/field. This approach was preferred because often there are focal variations in the epithelial lining as well as in the inflammatory infiltrate density within each cyst and proliferation may not be equally distributed along the lining. Therefore, analysis of data from each individual field is potentially more sensitive in detection of relationships between inflammation and expression of proliferation markers. The positively stained cells showed uptake of a brown color. PCNA and Bcl-2 positive cells were counted in 100 cells of each sample: 50 cells in the basal layer and 50 cells in suprabasal layer. Ten representative fields at 400 magnifications were selected and cells were counted in each of the mentioned layers. Thus, by selecting 10 random areas, which were not in the continuum with each other, a possible error in recounting the same cell was minimized.

Statistical analysis

The resulting data was analyzed using the Statistical Package for Social Sciences (SPSS) (SPSS, Chicago, IL, USA). Difference between the mean of two independent groups was observed by *t*-test if data is normally distributed. Differences between the different variables were analyzed using two-way analysis of variance (ANOVA) test and *post-hoc* test followed by Bonferroni test. $P < 0.05$ was considered as to be significant.

RESULTS AND DISCUSSION

The results showed that mild inflammation was observed in 2 cases of OKC and 9 of DC, moderate inflammation in 2 cases of OKC and 9 of DC and severe inflammation in 6 cases of OKC and 6 of DC. The presence of inflammation in OKC observed in our cases, may be partly due to possible communications with the oral mucosa via perforations of the cortical bone, which have been documented in up to 39% of OKC or it may also be introduced through the periodontal ligament in cases located close to adjacent teeth.[3] Inflammation in DC can be due to secondarily inflamed dental follicle or eruption of permanent successor into radicular cyst.[3] Table 1 represents the quantitative assessment of PCNA and Bcl-2 expression in basal and suprabasal cells of classical OKC, inflamed OKC, classical DC, inflamed DC [Figure 1] indicating that mean PCNA expression in suprabasal cell layers were significantly higher than basal cell layer of OKC and inflamed OKC [Table 1], which is also reported in previous studies.[3,4,6-8] This suggests that the highest proliferative activity is in the suprabasal cell layers, which reflects a unique epithelial differentiation process, in which the basal cells assume some characteristics of preameloblasts, which indicate that it might have entered to some extent toward ameloblast differentiation.[8] Piattelli *et al.* suggested that this could be due to inductive influences of the underlying connective tissue.[7] Inflamed OKC showed statistically significant increase in PCNA positive cells in both basal and suprabasal cell layers as compared with classical OKC by using the multiple bonferroni test [Table 2], which is in agreement with the previous studies,[4] but non-significant change was observed by Kaplan and Hirshberg.[3] Increased PCNA expression is suggestive of greater proliferative activity in the epithelial cells of inflamed OKCs, which could be associated with the disruption of the typical structure of OKC linings.[4,3]

Tables 1 and 2 reveal that more Bcl-2 expression was observed in the OKCs than DC with statistical significance ($P < 0.001$). Under the influence of inflammation both OKC and DC showed overexpression of Bcl-2, which suggested the increased survival of epithelial cells. [Figure 2] Hence,

Table 1: Quantitative assessment of PCNA and Bcl-2 expression in basal and suprabasal of four groups

Lesion	Layer	Mean±SD (PCNA)	Two-way ANOVA (*P* value)	Mean±SD (Bcl-2)	Two-way ANOVA (*P* value)
OKC	Suprabasal	219±45.8	*P*=0.001 (significant)	219±45.8	*P*=0.001 (significant)
	Basal	63.20±19.2		63.20±19.2	
Inflamed OKC	Suprabasal	279.8±34.86	*P*=0.001 (significant)	279.8±34.86	*P*=0.001 (significant)
	Basal	125.1±60.53		125.1±60.53	
DC	Suprabasal	108.1±31.78	*P*=0.001 (significant)	108.1±31.78	*P*=0.001 (significant)
	Basal	133.1±30.91		133.1±30.91	
Inflamed DC	Suprabasal	167.3±34.30	*P*=0.001 (significant)	167.3±34.30	*P*=0.001 (significant)
	Basal	221.4±57.09		221.4±57.09	

PCNA: Proliferative cellular nuclear antigen, SD: Standard deviation, OKC: Odontogenic keratocyst, ANOVA: Analysis of variance, DC: Dentigerous cyst

Table 2: Multiple comparisons between the four groups showing PCNA and Bcl-2 activity

Dependent variable	I and J Group	Mean difference (I-J) PCNA	*P* value (significant)	Mean difference (I-J) Bcl-2	*P* value (significant)
Suprabasal cells	OKC and I-OKC	−60.80	0.005	−4.20	0.0014
	DC and I-DC	−59.20	0.006	−2.90	0.005
	OKC and DC	110.90	0.001	8.50	0.001
	I-OKC and I-DC	112.50	0.001	10.30	0.001
Basal cells	OKC and I-OKC	−61.90	0.026	−6.20	0.06
	DC and I-DC	−88.30	0.001	2.30	0.001
	OKC and DC	−69.90	0.009	−24.60	0.001
	I-OKC and I-DC	−96.30	0.001	−27.90	0.001

PCNA: Proliferative cellular nuclear antigen, OKC: Odontogenic keratocyst, DC: Dentigerous cyst

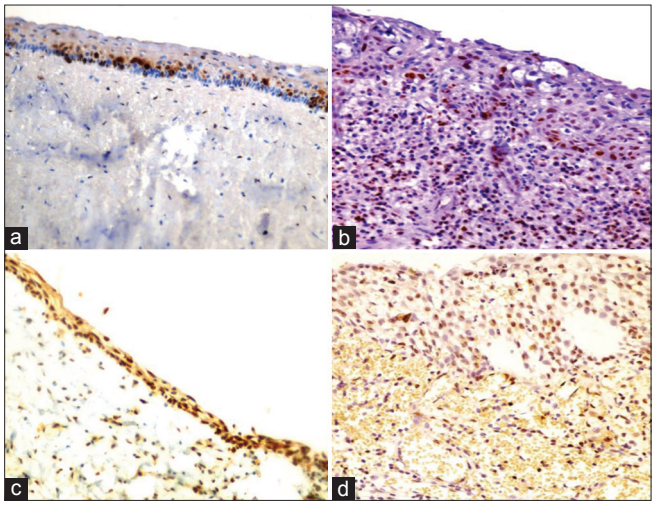

Figure 1: Proliferative cellular nuclear antigen immunostaining in (a) classical odontogenic keratocyst (OKC) (×40). (b) Inflamed OKC (×40). (c) Classical dentigerous cyst (DC) (×10). (d) Inflamed DC (×40)

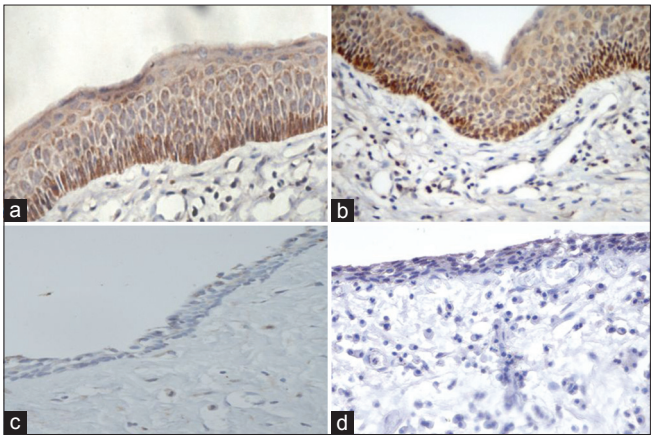

Figure 2: Bcl-2 immunostaining in (a) classical odontogenic keratocyst (OKC) (×40). (b) Inflamed OKC (×40). (c) Classical dentigerous cyst (DC) (×10). (d) Inflamed DC (×40)

we suggest that there is possible relationship between the aggressive nature of OKC and the intrinsic growth potential of its lining epithelium. Basal cell of OKC showed significantly higher expression Bcl-2 than DC, which can be useful for differentiating them from each other.[9]

DC showed higher expression in the basal layer than suprabasal layer, but it was statistically non-significant in classical DC and significant in inflamed DC [Table 1], which is in accordance with previous studies.[7,10] This suggests that the PCNA reactivity may be related to the regular maintenance of 2-3 cell layer thickness of the epithelium.[7] Thus, it advocated the mechanism of expansion of the DC that occurs passively by accumulation of fluid in the lumen rather than epithelial proliferation. Inflamed DC showed significantly higher PCNA expression in both basal and suprabasal layer cells as compare to classical DC [Table 2]. Martin *et al.*[10] also obtained similar results, but using the different proliferative marker Ki-67. Growth factors and cytokines are released during the inflammatory events, which increase cell proliferation.[6] The initial stimulus for the formation of DCs has not been clearly identified, but an inflammatory infiltrate in the cystic capsule has often been described. Therefore, we interpret our results as responses to inflammatory stimuli that may be the result of the eruptive process, may make cells proliferate, but may be inconstant and present for only short periods of time. This may explain the lower PCNA percentage found in DCs in this study. Their rate of proliferation is slower than that of OKC [Table 2], which justifies the aggressive behavior of OKC. The expression of markers may be more closely associated with cell stress caused by the inflammatory stimulus that leads to the formation of the cyst cavity. This cavity, once formed, initiates a feedback process due to the physiology of the epithelial tissue, which proliferates in the basal layer and desquamates into the cavity. The cyst becomes denser and thus attracts fluids to maintain its osmotic balance. Therefore, once the process initiates, it becomes independent of new inflammatory stimuli.[6]

Karl Pearson correlation test was applied, which showed a non-significant correlation between inflammation in relation to proliferative and anti-apoptotic activity in inflamed OKC and inflamed DC [Table 3]. From these results, it can be interpreted that there is non-uniform increase in PCNA and Bcl-2 expression with the increasing grades of inflammation. As inflammation has only secondary effect on the cystic lining, it would be more imperative that how the histogenetic precursors of these

Table 3: Correlation between inflammation and total PCNA and Bcl-2 expression in inflamed OKC and inflamed dentigerous cyst

Lesion	Pearson correlation coefficient (*r*)	
	PCNA expression	Bcl-2 expression (non- significant)
OKC	0.64 (significant)	0.33
Inflamed OKC	0.25 (non -significant)	0.15
Dentigerous cyst	0.33 (non- significant)	0.22
Inflamed DC	0.62 (non- significant)	0.25

PCNA: Proliferative cellular nuclear antigen, OKC: Odontogenic keratocyst

developmental cysts responds to such inflammatory stimuli. The role of inflammation and the sub-population of this inflammatory infiltrate could both be dependent and crucial for the plausible mechanism by which these apparatus would respond. The present study also leads us to probably understand the molecular pathways underlining the role of inflammation in the progression and behavioral changes of cyst. Antigenic intercepts causing lymphocytic activation are triggered from the cells of the epithelial lining. These antigenic targets put forth the recognition of the subjacent lymphocytes, which in turn through the mediation of its chemical messengers ignites a cascade of proliferative activity, which are primarily initiated in the basal layer.

CONCLUSION

Under the influence of inflammation over expression of Bcl-2 and PCNA suggested increased proliferation and survival of epithelial cells. Thus, we conclude that there is a possible relationship between the aggressive nature of OKC and the intrinsic growth potential of its lining epithelium. Inflammation is responsible for change in behavior of neoplastic epithelium of OKC, whereas in dentigeous it is responsible for changes in the epithelial lining.

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