A Comparative Immunohistochemical Expression of Cyclin D1 in Keratocystic Odontogenic Tumor, Dentigerous and Radicular Cysts

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ABSTRACT

Background: Odontogenic cysts include a group of osseodestructive lesions that frequently affect the jaws. Those cysts could derive from odontogenic epithelium and occur in the tooth-bearing regions of the jaws. The aims of this study were to evaluate the immunohistochemical expression of Cyclin D1 in Keratocystic Odontogenic Tumor, Dentigerous cyst and Radicular cyst in epithelium and connective tissue capsule.

Materials and Methods: In this study, thirty formalin fixed paraffin embedded tissue blocks of Odontogenic cysts and Tumor, consist of 14 Keratocystic Odontogenic Tumor, 8 dentigerous cysts and 8 radicular cysts were analyzed immunohistochemically for the presence of Cyclin D1 proteins.

Results: Strong to moderate expression of Cyclin D1 in epithelium was found in Keratocystic Odontogenic Tumor cases with positive cases percentage was (85.7%). Statistical significant differences (P<0.001) observed when comparing the three lesions. Immunoreactivity of Cyclin D1 in stroma of Keratocystic Odontogenic Tumor was higher than in dentigerous cysts and radicular cysts cases. However, the difference was not statistically significant (P<0.067).

Conclusion: The results of this study propose that high expression rate of Cyclin D1 might be one of the reasons for aggressive behavior of Keratocystic Odontogenic Tumor and high recurrence rate.

Key Words: Keratocystic Odontogenic Tumor, dentigerous cysts, radicular cysts, Cyclin D1, immunohistochemistry. (J Bagh Coll Dentistry 2016; 28(1):92-98).

INTRODUCTION

Odontogenic cysts are sub-classified as developmental or inflammatory in origin with different clinical and biological behaviors, odontogenic keratocyst (OKC) and dentigerous cyst (DC) are developmental in origin whereas, radicular cyst (RC) is an inflammatory in origin, which derived from the epithelial rest of malassez. Amongst these, odontogenic keratocyst demonstated aggressive behavior and higher recurrence rate [(1)].

Odontogenic keratocyst presents as a cystic structure similar to that of DC andRC, but its infiltrative and destructive growth is similar to that of ameloblastoma [(2)]. Several investigators have proposed that odontogenic keratocysts be regarded as benign cystic neoplasms rather than cysts, and in the latest WHO classification of Odontogenic tumors, these lesions have been given the term “keratocystic odontogenic tumor.” “KCOT” The arguments to support this change in nomenclature largely depend on a few studies that have shown certain molecular genetic alterations that are also present in some neoplasms, but little studies have analyzed other cystic lesions of the jaws; therefore, there is some confusion about these alterations whether are unique to the odontogenic keratocyst [(3)].

OKCs accounted for approximately 3-11% of all cysts in the jaws; when regarded as cyst. They occurred in all ages, with a peak incidence in the second and fourth decades of life. There was a slight male predilection [(4)]. KCOT can be found in the mandible and the maxilla but are twice as common in the mandible, with a predilection for the angle and ascending ramus [(5)].

DC were the most common developmental OC and comprised 18.1% of all OC [(6)]. DCs were discovered most frequently in patients between 10 and 30 years of age. There was a slight male predilection. DCs might occur in association with any unerupted tooth; most frequently mandibular third molars. Other moderately frequent sites included maxillary canines, maxillary third molars, and mandibular second premolars. Occasionally, they were associated with supernumerary teeth or odontomas. DCs rarely involved unerupted deciduous teeth [(7)]. There are some DCs reported to have a certain neoplastic potential manifested by ameloblatomatous transformation to form a unicystic ameloblastoma[(8)].

RCs represented the most frequently encountered jaw cyst 9. They constituted nearly one half to three fourths of all cysts in the jaws. The age distribution peaked in the third through sixth decades [(5)]. RCs were most common in the anterior maxillary area, especially in association with the lateral incisor teeth. RCs were more common in males than females [(6)]. Some cases of RC could grow and reach a considerable size, associated with local destruction [(8)].

Cyclin D1 is one of the Rb pathway proteins with oncogene properties which control G1-S transition. Elevated levels of this protein might permit cells to escape from the cell cycle check

point control and play an important role in tumorigenesis. Cyclin D1 is expressed in various types of malignant tumor whereas negative or weakly expressed in normal tissue and benign tumors [10]. Cyclin D1 served as a key sensor and integrator of extracellular signals of cells, mediating its function through binding both the CDKs and histone acetylase and histone deacetylases to modulate local chromatin structure of the genes that were involved in regulation of cell proliferation anddifferentiation [11]. The availablity of cyclin D1 was induced by growth factors including epithelial growth factor and Insulin Growth Factor (IGF) IandII; amino acids and hormones, each regulated expression of cyclin D1 in specific cell types [12,13].

Amplification or overexpression of cyclin D1 played key roles in the development of a subset of human cancers including parathyroid adenoma, breast cancer, lymphoma, melanoma, colon cancer, prostate cancer, head and neck Squamous Cell Carcinoma [14].

The aims of the present study were to evaluate the immunohistochemical expression of Cyclin D1 in keratocystic odontogenic tumor, Dentigerous cyst and Radicular cyst in epithelium and conecctive tissue capsule.

MATERIALS AND METHODS

In current study, thirty formalin fixed paraffin embedded tissue blocks of Odontogenic cysts , consist of 14 keratocystic odontogenic tumor, 8 dentigerous cysts and 8 radicular cysts were analyzed immunohistochemically for the presence of Cyclin D1 proteins. Positive staining indicates a lack of specification of the antibody and breast carcinomas were used as positive control for Cyclin D1 according to abcam manufacturer's data sheets.

Immunohistochemistry

Sections of 5µm were deparaffinized in xylene and rehydrated in graded alcohol. Enough drops of hydrogen peroxide block were added to slides and incubated in humid chamber at 37C° for 10 minutes, and then socked 2 times in buffer (5 minutes for each). Then tissue retrieving is done to the slides in order to uncover antigenicity because formalin or other aldehyde fixation forms protein cross-links that mask antigenic sites in tissue specimens. After that enough drops of protein block were added to slides and incubated at 37°C for 10 minutes. Then washed 2 times in buffer (5 minutes for each), finally drained and blotted gently. Then Diluted primary antibody was applied to each slide, incubated in humid chamber at 37°C. Overnight.

Early in the next day, the slides were washed in buffer (4 times for each), finally drained and blotted gently as before. Next enough drops of secondary antibody reagent were added and incubated in humid chamber for 30 minutes at 37°C. After that, the slides were washed 4 times in buffer (5 minutes for each), finally drained and blotted gently. Then Streptavidine-HRP antibodies were applied on tissue and incubated for 30 minutes at 37°C. Later Diluted DAB was applied on tissue (this process was done in dark room) and incubated in humid chamber for 10 minutes at 37°C. Then slides washed carefully in tap water for 5 minutes. After that the slides were bathed in Hematoxylin counterstain for 1-2 minutes then they were rinsed with tap water for 10 minutes. Later the slides were dehydrated by immersing them in ethanol and xylene containing jars then One to two drops of DPX mounting medium were applied to the xylene wet sections and covered with cover slips and left to dry overnight. Then the results were evaluated by the Presence of brown coloured end product at the site of target antigen (nucleus) was indicative of positive immunoreactivity. Percentage of IHC positive cystic cells per highlighting area was calculated and the mean percentage per slide was determined.

Assessing the expression of cyclin D1 in this study was classified as positive which showed brown nuclear staining in epithelial cells and connective tissue cells, whereas others were defined as negative. The positive cases were classified to additional categories, focal and diffuse expression. The slides were also evaluated for intensity of staining as mild, moderate and strong. Moreover, the epithelial layers predominantly containing the positive cells were noted in each group 1.

Immunoreactivity of Cyclin D1 was classified as follows into four categories according to the percentage of positively stained nuclei in the entire sections:

(score 0) (-ve) < 5% or less of epithelialand stromal cells positive.
(score 1) (+) 5-25%.
(score 2) (++)> 25-50%.
(score 3) (+++)> 50 % or more of epithelial and stromal cells positive 15.

Statistical Analysis

All the clinical, histopathological and immunohistochemical relevant data so obtained was tabulated and subjected to appropriate statistical analysis using the SPSS 17 statistical software. Numerical values were used in this study for describing the variables which includes:

Median, maximum, minimum and mean ± SD for age, Cyclin D1 protein expressions.

Categorical variables which include: sites, sex and clinical presentation were described using Number and percentage. Non parametric Chi-square test was used to evaluate the difference between the scores. Kruskall-Wallis H test used for ordinal variable followed by Mann-Whitney U test was used to find the relation between two cystic lesions. P value equal or less than 0.05 was considered to be statistically significant.

RESULTS

Cyclin D1 immunoreactivity was noticed as brown staining localized in the nucleus of both epithelial and mesenchymal cells (figures 1 to 6). Strong to moderate expression of Cyclin D1 in epithelium was found in odontogenic keratocysts cases with positive cases percentage was (85.7%).Statistical significant differences (P<0.001) observed when comparing the three lesions. Immunoreactivity of Cyclin D1 in stroma of keratocystic odontogenic tumor was higher than in dentigerous cysts and radicular cysts cases. However, the difference was not statistically significant (P<0.067).

Table 1: Age, Sex, and Site Distribution for Cases with KCOT, DC and RC of the Jaw

Variable	KCOT(14)	DC (8)	RC(8)
Age			
Mean±SD	30.36±13.80	24.50±17.26	29.50±20.38
Minimum	12	5	11
Maximum	57	50	63
Sex			
Male	10(71.43%)	4(50%)	5(62.5%)
Female	4(50%)	4(50%)	3(37.5%)
Site			
Maxilla	5(35.71%)	6(75%)	7(87.5%)
Mandible	9 (64.29%)	2(25%)	1(12.5%)

Table 2: Cyclin D1 Score Distribution in the Cystic Epithelium of KCOT, DC and RC Cases

Scores	Lesions		
	KCOT 14	DC 8	RC 8
0	2 (14.29%)	4(50%)	5(62.5%)
1	0(0%)	3(37.5%)	0(0%)
2	6 (42.86%)	1 (12.5%)	3 (37.5%)
3	6(42.86%)	0(0%)	0(0%)
Test	Chi Square=20.328 ; P-Value= 0.001(HS)		

Table 3: Case Distribution of Odontogenic Cysts According to Expression Pattern of Cyclin D1

Expression Pattern	Lesions		
	KCOT (12)	DC(4)	RC(3)
Diffuse	4(33.33%)	2(50%)	2(66.66%)
Focal	8(66.67%)	2(50%)	1(33.33%)
Test	X2=1.162; p-value=0.558(NS)		

Table 4: Case Distribution of Odontogenic Cysts According to Staining Intensity of Cyclin D1

Staining Intensity	Lesions		
	KCOT (12)	DC(4)	RC(3)
Mild	1(8.33%)	1(25%)	1(33.33%)
Moderate	4(33.33%)	3(75%)	2(66.66%)
Strong	7(58.33%)	0(0%)	0(0%)
Test	X2 =7.485 ; p-value = 0.054(NS)		

Table 5:Case Distribution of Odontogenic Cysts According to Staining Localization of Cyclin D1

Staining Localization	Lesions		
	KCOT(12)	DC(4)	RC(3)
All layer	0	1	0
	(0%)	(100%)	(0%)
Basal and/or parabasal	2	1	1
	(50%)	(25%)	(25%)
Parabasal	9	2	2
	(69.2%)	(15.4%)	(15.4%)
Parabasal and superfacial Layer	1	0	0
	(100%)	(0%)	(0%)
Test	X2 =4.963; p-value= 0.588(NS)		

Table 6: Cyclin D1 Score Distribution in the Connective Tissue Capsule of KCOT, DC and RC Cases

Scores	Lesions		
	KCOT(14)	DC(8)	RC(8)
0	3(21.43%)	3(37.5%)	4(50%)
1	1(7.14%)	3(37.5%)	3(37.5%)
2	7 (50%)	2(25%)	1(12.5%)
3	3(21.43%)	0(0%)	0(0%)
Test	X2 =10.056 ; p-value=0.067(NS)		

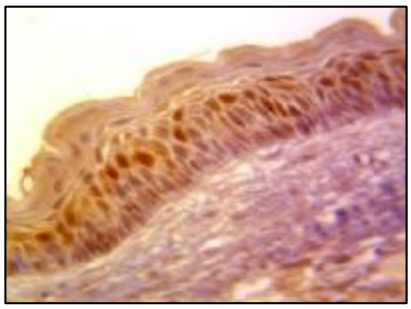

Figure 1: Keratocystic Odontogenic Tumor: Cyclin D1 IHC Expression of Cystic Epithelium with Strong Intensity and Focally Pattern with Immunoreactivity in Basal and Parabasal Layer X400.

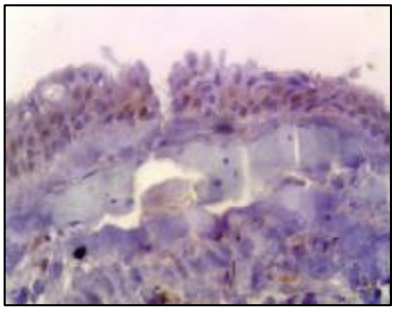

Figure 2: Dentigerous Cyst: Cyclin D1 IHC Expression of Cystic Epithelium with Moderate Intensity and Focally Pattern with Immunoreactivity in Basal and Parabasal Layer X400.

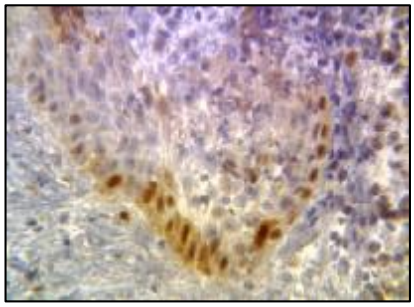

Figure 3: Radicular Cyst: Cyclin D1 IHC Expression of Cystic Epithelium with Moderate Intensity and Focally Pattern with Immunoreactivity in Basal Layer X400.

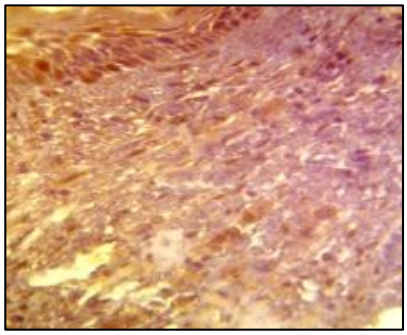

Figure 4: Keratocystic Odontogenic Tumor: Cyclin D1 IHC Expression of the Connective Tissue Capsule X400.

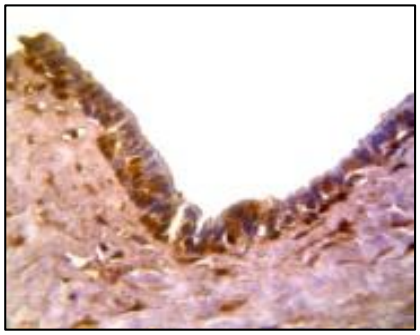

Figure 5: Dentigerous Cyst: Cyclin D1 IHC Expression of the Connective Tissue Capsule X400.

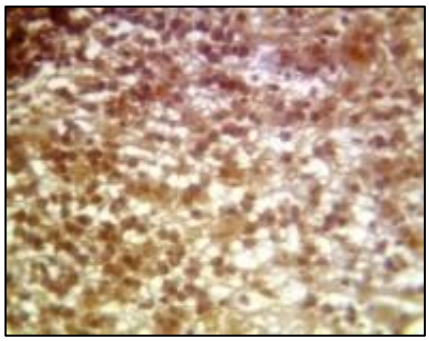

Figure 6: Radicular Cyst: Cyclin D1 IHC Expression of the Connective Tissue Capsule X400.

DISCUSSION

The transition between different cell cycle stages is regulated at several checkpoints. Regulation of the G1-S transition is controlled by the Rb pathway proteins, which include, among others, the cyclin D1 gene. The over expression of this protein shows accelerated G1 progression entering in the S-phase of cell cycle. Cyclin D1 may play an important role in tumorigenesis and it has been detected in malignant tumors but also in benign neoplasms [16].

In the present study, 12 of 14(85.7%) cases of Keratocystic Odontogenic Tumor, 4 of 8 of Dentigerous cysts (75%) and 3 of 8 of Radicular cysts(62.5%) cases showed positive immunoreactivity to Cyclin D1.

This is in line with many studies [15-17]. In their study de Vicente et al., cyclin D1 was found in 91% of KCOTs, and the rate of its expression was significantly higher than that of DC (50%), RC (40%). They observed the cyclin D1 expression in a focal and basal pattern, while in DCs and in RCs focal and diffuse patterns were equally observed. The staining pattern was only diffuse. Razavi et al. found in their study the expression of cyclin D1 in the suprabasal layers of KCOTs was significantly higher than in the basal and superficial layers ($P < 0.001$).

Taghavi et al. observed a decreased staining positivity for cyclin D1 in the following order: KCOTs (87%), GOCs (60%), RCs (30%), and DCs (25%). It was detected mostly in the parabasal layer of all cysts types. Kimi et al. investigated immunohistochemical expression of cyclin D1 and p16 in sporadic, recurrent and syndromic KCOTs. Similar to the present study, cyclin D1 was detected in parabasal layer of KCOTs with higher expression in syndromic KCOTs. Lo Muzio et al., [18] compared cyclin D1 expression in sporadic KCOTs and KCOTs associated with Gorlin syndrome. On the contrary, they observed cyclin D1 expression just in syndromic KCOTs not in sporadic ones. The discrepancy between findings may be due to various laboratory methods and used antibody.

Evaluation of staining pattern and intensity in the present study showed no significant difference among groups. This result is in agreement with De Vicente et al., [16] who examined cyclin D1 expression in OKCs in comparison with other lesions including ameloblastoma.

In most KCOTs, cyclin D1 was detected in parabasal layer and in consistent with other studies [1,15,16]. These results may imply different proliferative activity in the epithelial layers in each group. Besides, it can justify the aggressive behavior of KCOTs.

The increased cellular proliferation in the suprabasal layer of the KCOTs may suggest an abnormal cell cycle control. This unusual pattern of proliferation in the KCOTs lining is also thought to represent an epithelial disorganisation similar to dysplasia of oral squamous epithelium in premalignant lesions of the oral mucosa, whereby proliferating cells are found in greater numbers in the suprabasal compartment [19-22]. The high proliferative potential of the KCOTs also reinforces its classification as a benign cystic odontogenic neoplasm [23].

Till the time of the present study, there was no study in the literature established to examine Cyclin D1 expressions in connective tissue cells of KCOTs, DC and RC, so the present study is important to compare connective tissue cells of KCOTs, DC and RC.

There was emphasis in research which concentrated on the epithelium itself, only a few studies have investigated non-epithelial factors i.e. underlying connective tissue stroma that could contribute to the variable biological behavior of the different types of odontogenic cysts and tumors and needs more investigation. Hirsberg et al., [24] made a Study on the KCOTs connective tissue. They revealed that stroma not only plays a supporting structure role, but also has an important role in its neoplastic behavior.

In the present study, 11 of 14(78.57%) cases of KCOTs, 5 of 8 of DC (62.5%) and 4 of 8 of RC (50%) cases showed positive immunoreactivity to Cyclin D1 in stroma. So positive connective tissue cells for Cyclin D1 were recognized exclusively in KCOTs, whereas other two groups expressed very low Cyclin D1 positive cells in their stroma. This reflects the intrinsic growth potential of KCOTs which was different from other cysts and the stroma played a role in their aggressive behavior.

TekkeşİN et al.,[25] showed that positive connective tissue cells for Ki-67 were recognized exclusively in RC, whereas KCOTs and AB expressed very low Ki-67 positive cells in their stroma. ($p<0.01$, $p<0.09$, respectively) There was no significant difference in the Ki-67 expression between connective tissue cells of KCOTs and AB. As explanation of their study these features may suggest that heavy subepithelial chronic inflammation of RC can stimulate proliferation of fibroblasts and endothelial cells. Regarding Ki-67 as a proliferation markers used in this study, this study disagreed with results of the present study in regarding KCOTs and RC. The reasons of this discrepancy may be 1st difference in markers used, 2nd limited number of sample in the present study.

The results of this study propose that high expression rate of Cyclin D1 might be one of the reasons for aggressive behavior of Keratocystic Odontogenic Tumor and high recurrence rate.

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