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Comparison of β-catenin Expression in Keratocystic Odontogenic Tumor, Dentigerous Cyst and Ameloblastoma

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A B S T R A C T

Odontogenic keratocyst is now designated by the World Health Organization as a keratocystic odontogenic tumor (KCOT). According to recent reports Wnt pathway and ß-catenin are involved in the pathogenesis of the majority of odontogenic and neoplastic lesions. The aim of this study was to evaluate the expression of ß-catenin in KCOT and compare it with those of dentigerous cyst and ameloblastoma. Expression of ß-catenin was investigated by immunohistochemistry using specific monoclonal antibodies in 57 formalin-fixed paraffin-embedded samples of KCOT, dentigerous cyst and ameloblastoma (19 samples in each group). The expression of β-catenin protein was observed separately in the membrane, cytoplasm and nucleus of epithelial cells. Percentages of expression of membranous, cytoplasmic, and nuclear ß-catenin were 68.4%, 89.5% and 5.3% for KCOT; 78.9%, 100%, and 26.3% for ameloblastoma and 42.1%, 84.2%, and 5.3% for dentigerous cyst, respectively. The expression of membranous ß-catenin in the dentigerous cyst was significantly lower than that in KCOT and ameloblastoma ($P<0.05$). In other cases, there were no significant statistical differences between the three groups. Membranous expression of ß-catenin might be useful in differentiation of KCOT and ameloblastoma from dentigerous cyst. However, intracellular accumulation of this protein cannot be used to prove the neoplastic nature of KCOT versus dentigerous cyst. Moreover, considering the intracellular accumulation of ß-catenin in most cases in all the three lesions, it seems that ß-catenin changes might play a role in development of odontogenic epithelium in this lesion through deregulation of cell cycle.

Introduction

Odontogenic keratocyst is a distinctive form of developmental odontogenic cysts; it has drawn a great deal of attention because of its clinical behavior and specific

histopathologic features (Neville and Damm 2008). This lesion represents approximately 5□15% of all the odontogenic cysts. Most authors believe it originates from the dental lamina remnants in the maxilla and mandible and consider high proliferation rate, overexpression of Bcl2 and expression of matrix metalloproteinases (MMP_S 2 and 9) as the pathogenetic mechanisms for its growth and expansion (Regezi *et al.,* 2008). Recently, some researchers have suggested that odontogenic keratocyst should preferably be considered a benign cystic neoplasm rather than a cyst and in the final classification of odontogenic tumors by the World Health Organization (WHO), these lesions have been given the name keratocystic odontogenic tumor (KCOT). Currently, there is not enough definitive evidence for the neoplastic nature of this lesion; furthermore, sufficient research has not been carried out on other common odontogenic cysts. There is controversy over this issue in the professional community (Neville and Damm 2008).

Moreover, Wnt-signaling plays an essential role in a number of developmental processes and tumorogenesis (Kim *et al.,* 2007). Roles for Wnt have been recognized in three distinct pathways. One of the pathways includes adenomatous polyposis coli protein (APC), Axin1, and β-catenin (Siriwardena *et al.,* 2009). In cell nucleus, β-catenin interacts with transcription factors and increases cell proliferation by increasing transcription of target genes. Nuclear and cytoplasmic accumulation of β-Catenin is created through deactivation of APC or Axin1 or mutations in β-Catenin molecule or activation of Wnt-signaling pathway. It is conceivable that β-catenin acts as an oncogene product (Kumar *et al.,* 2010). Mutations in β-catenin gene are reported in the colon and gastrointestinal tract cancers, desmoid tumor, medulloblastoma, melanoma, ovarian cancer, pancreatic and prostate tumors including ghost cell, pilomatricoma and craniopharyngioma (Kim *et al.,* 2007; Siriwardena *et al.,* 2009).

Wnt signaling pathway plays an essential role in tooth development and directs multiple stages of tooth morphogenesis. Tight regulation of this pathway is essential for both patterning tooth development in the dental lamina and controlling the shape of individual teeth (Sarkar and Sharpe, 1999; Liu *et al.,* 2008).

Several studies have also examined the expression of these proteins in odontogenic cysts and tumors, reporting conflicting results (Kim *et al.,* 2007; Siriwardena *et al.,* 2009; Sekine *et al.,* 2003; Rosai and Akerman, 2004; Ahn *et al.,* 2008). Ameloblastoma and KCOT have both benign but locally aggressive behavior; however, they have a high risk of recurrence (da Silva *et al.,* 2008). Due to the high expression of β-catenin in ameloblastoma and calcifying odontogenic cyst (COC), in this research the expression of β-catenin in KCOT was compared with those of ameloblastoma and dentigerous cyst by using immunohistochemistry.

Materials and Methods

A total of 19 formalin-fixed paraffin-embedded tissue samples of KCOT, 19 dentigerous cysts and 19 ameloblastoma from the archives of the Department of Oral and Maxillofacial Pathology, Tabriz University of Medical Sciences, were examined. Information including age, sex and location were extracted from patient records. Microscopic slides of selected samples were stained by hematoxylin & eosin and were reviewed by two oral pathologists to confirm the previous diagnosis. Sections measuring 5 μm were

cut; deparafinized and immune-histochemicaly stained using Dako Envision system from the same paraffin-embedded samples whose diagnoses were confirmed by oral pathologists. Sections were treated with Anti-b-catenin (Dako, Denmark, Code M3539) as primary antibody at a dilution of 1:100 (Dako, Denmark, Code S0809) for 60 minutes at room temperature and incubated at 4°C overnight. Primary antibody was visualized with diaminobenzidine (DAB Chromagen; Dako). Tissue sections of oral squamous cell carcinoma were also stained as positive controls. For the negative control, PBS was applied in order to substitute the specific antibody. Examination of slides from each specimen was carried out on an Olympus U-MDOB light microscope at ×400 magnification. Pictures were obtained by a color video camera (JVC, TK-C1380). Expression of β-catenin protein was observed separately in the membrane, cytoplasm and nucleus of epithelial cells. Expression of β-catenin was considered negative where less than 30% of epithelial cells of the lesions were negative and positive if it was more than 30% (Siriwardena *et al.,* 2009). Chi-squared and Fisher's exact tests were used to evaluate data. Statistical significance was defined at $P \leq 0.05$. Statistical analyses were carried out using SPSS 15 statistical software program.

Result and Discussion

The expression of β-catenin was demonstrated as brown color after immunohistochemical staining (Figure 1). The percentages of expression of membranous, cytoplasmic, and nuclear ß-catenin were 68.4%, 89.5% and 5.3% for KCOT; 78.9%, 100%, and 26.3% for ameloblastoma and 42.1%, 84.2%, and 5.3% for dentigerous cyst, respectively. The expression of membranous ß-catenin in the dentigerous cyst was significantly lower than those of KCOT and ameloblastoma ($P<0.05$). In other cases, there were no statistically significant differences between the three groups ($P>0.05$). The studied variables in the three groups are presented in Table 1.

Keratocystic odontogenic tumor has recently been suggested to describe the lesion previously named odontogenic keratocyst (Neville and Damm 2008).

Considering the recent reports as to the involvement of Wnt/β-catenin signaling pathway in the pathogenesis of most neoplastic lesions (Kim *et al.,* 2008), the expression of ß-catenin was evaluated in KCOT and compared with those in dentigerous cyst and ameloblastoma. ß-catenin is one of the key elements of Wnt-signaling pathway. Accumulation of β-catenin in the nucleus and cytoplasm is a result of deactivation of APC or Axin1 or mutations in β-catenin molecule or activation of Wnt-signaling pathway that can be used as a useful marker to detect cellular conditions (Siriwardena *et al.,* 2009).

Previously, the results of three studies by Hassanein, Ahn and Kim have shown the expression of β-catenin in COC samples (that are classified by WHO as odontogenic tumors). Therefore, Wnt pathway alternations might play a crucial role in the development and differentiation of odontogenic epithelium in these lesions through deregulation of cell proliferation (Kim *et al.,* 2008; Ahn *et al.,* 2009; Hassanein *et al.,* 2003). This is one of the reasons for carrying out the present study.

The present study is the first study investigating the expression of β-catenin in KCOT as the main element of the Wnt-

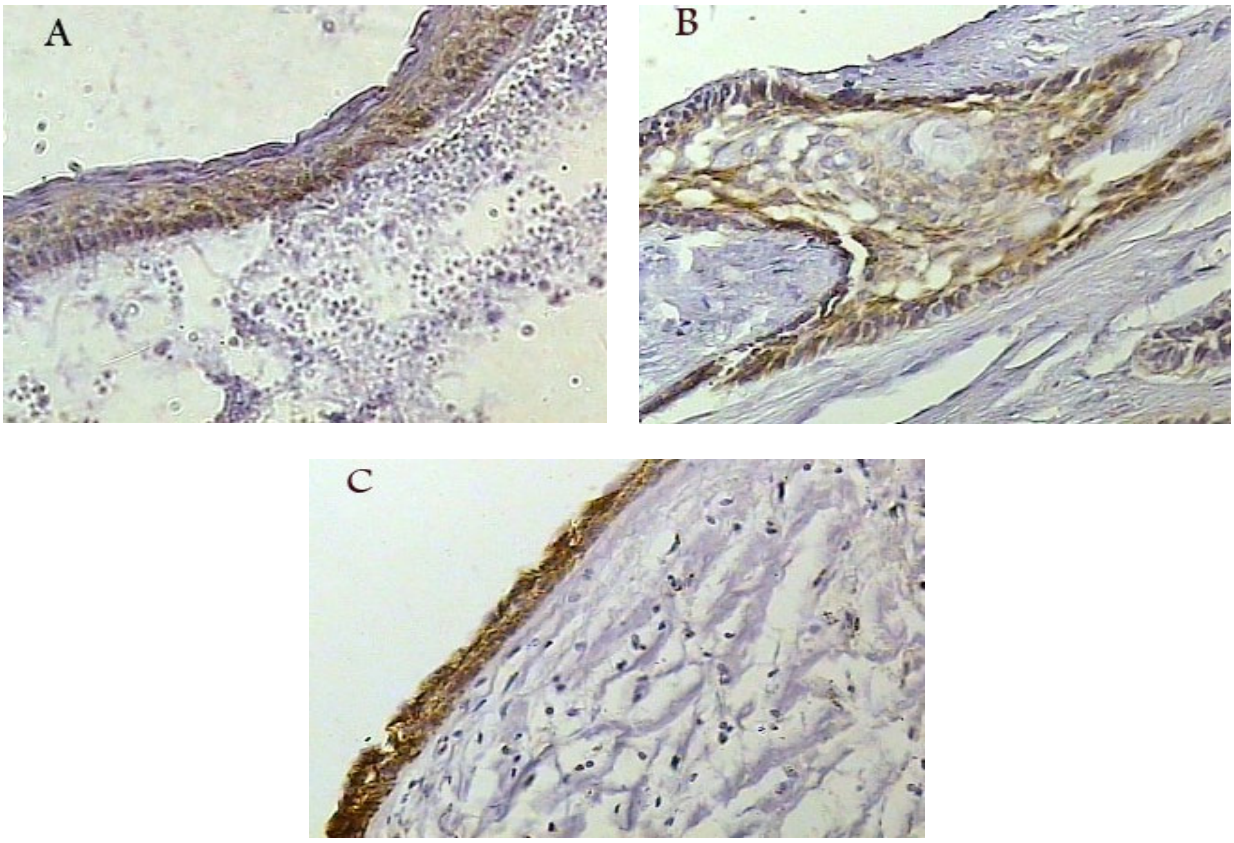
signaling pathway to compare it with those of ameloblastoma and dentigerous cyst.

Sekine *et al.* (2003) compared COC and ameloblastoma samples in terms of mutations in the β-catenin gene and expression level. They concluded that nuclear and cytoplasmic expressions have a heterogeneous pattern in all the COC samples.

Table.1 Clinical characteristic of keratocystic odontogenic tumor, ameloblastoma and dentigerous cyst

Variable		Keratocystic odontogenic tumor (n=19)	Ameloblastoma (n=19)	Dentigerous cyst (n=19)	P-value
Age		36.26±14.76 (17-70)	44.47±18.53 (10-71)	23.21±8.34 (9-42)	-
Sex	Male	14(73.7%)	11(57.9%)	7(36.8%)	-
	Female	5(26.3%)	8(42.1%)	12(63.2%)	
Place	Mandible	14(73.7%)	15(78.9%)	11(57.9%)	-
	Maxilla	5(26.3%)	4(21.1%)	8(42.1%)	
β-Catenin	Membranous	13(68.4%)	15(78.9%)	8(42.1%)	0.05
	Cytoplasmic	17(89.5%)	19(100%)	16(84.2%)	0.55
	Nucleus	1(5.3%)	5(26.3%)	1(5.3%)	0.41

Figure.1 Immunohistochemical staining of β-catenin in keratocystic odontogenic tumor (A), ameloblastoma (B) and in dentigerous cyst (C)



Expression of β-catenin was moderate in the nucleus and cytoplasm of the follicular ameloblastoma samples and in the membrane of the plexiform types. In contrast, in this study, the expression of membranous and cytoplasmic β-catenin was higher in the ameloblastoma samples and nuclear staining was lower.

Miyake *et al.* (2006) investigated the immunohistochemical expression of β-catenin and the related frequency of gene mutations in ameloblastoma and malignant odontogenic tumors. They suggested that mutation of this gene is not frequent in ameloblastoma. However, the Wnt pathway disorders might be involved in the pathogenesis of some odontogenic lesions.

The results of the present study are consistent with those reported by Kumamoto and Ooya (2005) in membranous, cytoplasmic and nuclear staining of ameloblastoma. Consequently, although in the present study the increased expression of β-catenin in the nucleus of ameloblastoma samples was not statistically significant, in a recent study by Siriwardena (2009) in all the cases of ameloblastoma and 75% of cases of odontogenic carcinoma, accumulation of β-catenin was present in the nuclei of tumor cells. These researchers concluded that unusual accumulation of β-catenin might play an important role in the pathogenesis of odontogenic epithelial tumors.

In the normal state, β-catenin binds to the cell membrane as a part of the membrane-bound cadherin-catenin complex (Hassanein *et al.*, 2003). The association of the cytoplasmic domain of cadherin with the actin cytoskeleton via catenin is critical for cell-cell adhesion.

Loss of epithelial integrity due to abnormal expression of E-cadherin, α-catenin, or β-catenin has been shown in a number of tumors. It has been postulated that the abnormal expression or dysfunction of any component of this complex might contribute to the malignant phenotype (Osterheld *et al.*, 2002).

Considering the possibility of carcinomatous transformation in dentigerous cysts (Neville and Damm, 2008), the low expression of membranous ß-catenin in dentigerous cysts might be attributed to malignant potential of the lining of this lesion.

In the present study cytoplasmic expression of β-catenin was high in the three lesions under study, but nucleus accumulation of protein was observed in some samples. In normal state there is no β-catenin in large quantities in the cytoplasm or nucleus of cells (Kumamoto and Ooya, 2005). Replacement of the protein in a place other than that of cell membranes might be related to translocation to the nucleus where it activates transcription (Rodriguez-Pinilla *et al.*, 2005).

In general, based on current study results, due to the cytoplasmic accumulation of β-catenin, it seems that β-catenin changes might play a role in the pathogenesis of these lesions through deregulation of cell cycle. Membranous β-catenin might be involved in distinguishing two groups of KCOT and dentigerous cyst and also in distinguishing two groups of ameloblastoma and dentigerous cyst.

However, accumulation of intracellular protein cannot be used to prove the neoplastic nature of KCOT. It seems that more studies in this field are necessary to identify specific genetic mutations in odontogenic lesions. In fact this study was only a preliminary study in this regard and further studies should be carried out.

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