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Original Article

Immunohistochemical evaluation of podoplanin in odontogenic tumours & cysts using anti-human podoplanin antibody

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ABSTRACT

Background: Odontogenic Cysts & tumors originate through some aberration from the normal pattern of odontogenesis. Ameloblastoma is one of the most frequent intraosseous odontogenic tumors. However it is no longer appropriate to use the diagnosis of ameloblastoma without specifying the type. Varied-clinical entities of ameloblastoma differ in their biologic behaviour. Odontogenic cysts like dentigerous and radicular cysts are less aggressive in nature than odontogenic tumors. Recently, **podoplanin** commonly used as a lymphatic endothelial marker in cancers has recently been found to play a possible role in odontogenic tumorigenesis also. Therefore the purpose of this study was to immunohistochemically analyse the expression of podoplanin in ameloblastomas, KCOTs, dentigerous cysts, radicular cysts & dental follicles.

Methods: Paraffin-embedded tissue specimens of 15 Ameloblastomas (7 follicular, 6 unicystic, 2 desmoplastic),10KCOTs, 5 dentigerous cysts, 5 radicular cysts & 5 dental follicles were immunohistochemically examined using antibody against podoplanin.

Results: All ameloblastomas displayed podoplanin expression in ameloblast-like cells of the epithelial islands while the stellate-reticulum like cells exhibited no or weak immunostaining. Expression of podoplanin in KCOTs was strongly positive in the cells of the basal and suprabasal layers & odontogenic epithelial nests. Positive immunoreaction for podoplanin was observed in the inflammatory radicular cysts and inflamed dentigerous cyst only and negative or weak expression in the lining epithelium of uninflamed dentigerous cysts and dental follicles.

Conclusion: Our results suggest that podoplanin can be used as a potential proliferative marker to observe the aggressive behaviour of ameloblastomas and KCOTs.

1. Introduction

A multitude of odontogenic tumors and cysts occurring in the oral and maxillofacial region originate through some aberration from the normal pattern of odontogenesis which reflect their complex multiformity.[1] It is no longer appropriate to use the diagnosis of ameloblastoma which is the second most common odontogenic neoplasm without specifying the type in any scientific study.Varied-clinical entities of ameloblastoma differ in their biologic behaviour. The solid / multicystic ameloblastoma is a slowly growing, locally invasive, epithelial odontogenic tumour of the jaws with a high rate of recurrence. The unicystic ameloblastoma on the other hand presents as a cyst with low recurrence rate & less aggressive behaviour. Recently desmoplastic variant of ameloblastoma is also regarded as a separate clinical entity based on its distinct clinicopathologic behaviour.[2] The odontogenic keratocyst (OKC) is known to have a more aggressive biologic behavior than other cysts of jaw. In the 2005 WHO classification, odontogenic keratocyst has been redesignated under the name of keratocystic odontogenic tumor (KCOT). Hence in this study this lesion is referred to as KCOT. These benign odontogenic tumors

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have the property of invasion, resulting in high recurrence rate. The extent of invasion can be analysed by the expression and production of various genes and proteins by lesional cells. However, current clinical parameters lack the potential to predict the neoplastic behavior in KCOTs. Till date, no suitable immunohistochemical marker is available to assess the aggressiveness of KCOT.

Podoplanin which is frequently used as a lymphatic endothelial marker in OSCCs has recently been found to play a possible role in odontogenic tumorigenesis also. Podoplanin is a 38 kDa type-1 transmembrane sialomucin -like glycoprotein which consists of 162 amino acids. In the recent literature, podoplanin expression is also observed in odontogenic tissues like in secretory ameloblasts, developing & mature odontoblasts, Tomes' fibres & pulp cells.3

The podoplanin expression in tumorous odontogenic cells is a recent topic of interest. Both mitotic activity and podoplanin expression within the ameloblastoma are coincident (i.e., restricted to the peripheral epithelial cells of the tumor cords and strands). This pattern of distribution of podoplanin immunostaining, according to the cellular subtype in ameloblastomas, may be helpful to the classification of odontogenic tumors.4

Various clinical and laboratory studies reviewed in the past provided evidence of the distinctively less aggressive nature of the dentigerous & radicular cysts than odontogenic keratocysts. The expression of podoplanin in these odontogenic cysts can therefore be compared with that of KCOTs. Moreover, the question of whether inflammation in dentigerous cyst might influence its behaviour & may bring about a change in the immunohistochemical expression of podoplanin in the developmental cyst can also be evaluated.

Podoplanin has been also found to modulate the actin cytoskeleton, thereby suggesting an important role in tumor invasion and metastasis.5 It may be involved in the process of local expansion of developmental, inflammatory and neoplastic odontogenic lesions. The pattern of staining for podoplanin in KCOT could be related to its neoplastic nature, and may suggest a role of this protein in tumor invasiveness.

Therefore, this study aims to analyse the expression of podoplanin in the ameloblastomas and keratocystic odontogenic tumors (KCOTs), further to elucidate and reinforce the importance of this molecule in the growth of these tumors & to compare their pattern of expression with dentigerous & radicular cysts.

1.1. Materials & methods

The paraffin embedded study specimens which included fifteen cases of Ameloblastomas (seven follicular, six unicystic & two desmoplastic), ten cases of Keratocystic Odontogenic Tumors, ten cases of odontogenic cysts (five dentigerous cysts & five radicular cysts) & five sections of dental follicles (taken as control) were retrieved from the archives of Department of Oral & Maxillofacial Pathology, Kanti Devi Dental College, Mathura, U.P.

2. Immunohistochemistry

Deparaffinized sections were immersed in 0.01 M citrate buffer, pH 6.0, and heated in a microwave oven for 5 min at high voltage and then for 15 min at low voltage for antigen retrieval. The tissue was then immersed in peroxide block for 20 min at room temperature to block endogenous peroxidase activity. After PBS washing, tissue was covered with power block reagent for 20 minutes. Ready to use mouse monoclonal anti-human D2–40 (anti-podoplanin) antibody(Dako Flex)was applied to the sections for 1 h at room temperature and the slides were then covered with Post primary Block for 25 minutes.The sections were then covered with secondary antibody conjugated with peroxidise.This was followed by application of freshly prepared substrate chromogen (DAB) solution for 2 minutes and then counterstained with Mayer's hematoxylin.

3. Immunostaining evaluation

Scoring was based on:

Intensity of the podoplanin expression in the epithelial odontogenic Cells (A): 0 = absent, 1 = weak, 2 = moderate,3 = strong, and 4 = very strong.

Percentage of podoplanin positive odontogenic cells (B): 0 = 0% positive cells,1 = <25% positive cells,2 = 25–50% positive cells,3 = 50–75% positive cells,4 = >75% positive cells.

Final score (A + B): 0 = absent,1–4 = weak, and 5–8 = strong.

Final scores ranged from 0 to 8: (0 = absent, 1–4 = weak,5–8 = strong).

For statistical analysis,the independent t- test was used to compare the mean scores among the study groups. The level of significance was set at 5% for all tests.

4. Results

The immunohistochemical results for Ameloblastomas, KCOTs, Dentigerous cysts, Radicular cysts & Dental Follicles are summarised in Table 1. The immunohistochemical results for odotogenic tumors, odontogenic cysts & dental follicles are given in Table 2. The strong expression of podoplanin in follicular ameloblastomas was predominantly observed in the peripheral columnar cells of

Table 1
Showing the t-values & p-values of the intensity of the Podoplanin immunostaining(A), Percentage of Podoplanin positive odontogenic cells(B) & the Final immunostaining score(A + B) comparing between 15 cases of Ameloblastomas,10 cases of KCOTs,5 cases of Dentigerous cysts, 5 cases of Radicular cysts & 5 cases of Dental Follicles.

Group	Immunostaining Intensity (A)		% of Podoplanin Positive Cells (B)		Total (A + B)	
	t-value	p- value	t-value	p-value	t-value	p-value
Ameloblastomas vs KCOTs	0.335	>0.05(ns)	1.515	>0.05(ns)	0.473	>0.05(ns)
Ameloblastomas vs Dentigerous cysts	3.623	<0.05	3.964	<0.05	4.157	<0.05
Ameloblastomas vs Radicular cysts	1.767	>0.05(ns)	1.699	>0.05(ns)	1.938	>0.05(ns)
Ameloblastomas vs Dental Follicles	3.797	<0.05	5.196	<0.05	4.838	<0.05
KCOTs vs Dentigerous Cysts	4.869	<0.05	3.726	<0.05	5.256	<0.05
KCOTs vs Radicular Cysts	2.757	<0.05	0.745	>0.05(ns)	2.360	<0.05
KCOTs vs Dental Follicles	5.513	<0.05	5.507	<0.05	6.687	<0.05
Dentigerous Cysts vs Radicular Cysts	2.631	<0.05	2.581	<0.05	2.840	<0.05
Dentigerous Cysts vs Dental Follicles	0.000	>0.05(ns)	1.414	>0.05(ns)	0.631	>0.05(ns)
Radicular Cysts vs Dental Follicles	3.953	<0.05	4.242	<0.05	4.348	<0.05

ns: not significant, p -value set at 5%.

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