Role of Podoplanin in Progression and Invasive Behavior of Odontogenic Cysts and Tumour: An Immunohistochemical Evaluation

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Abstract

Background: Dentigerous cysts are developmental in origin while periapical/radicular cyst is due to a long term inflammatory process in the bone of the surrounding root apex. The odontogenic keratocyst arises from the dental lamina and other sources of odontogenic epithelium and aggressive in its growth pattern, with a higher recurrence rate. Podoplanin has been considered as a specific marker for lymphatic endothelial cells. Its expression can be observed in odontogenic epithelial and mesenchymal tissues. Recently, it has been found that it plays a possible role in odontogenic tumorigenesis also.

Methodology: We took n=44 cases comprising of dentigerous cyst (n=04), odontogenic keratocysts (n=11), periapical cysts (n=15) and ameloblastoma (n=14). Relevant clinical and radiographical findings were recorded and biopsies were submitted for histological diagnosis. Podoplanin immunopositivity was assessed by immunohistochemistry.

Results: Among n=44 cases of odontogenic cysts and tumors mean age was 29.6 ± 13.05 years and 27.2 ± 7.93 years for the developmental and inflammatory odontogenic cysts (radicular cyst) respectively while 42.5 ± 9.28 years for ameloblastoma. Male predominance was seen in all cases. Radiographically, mostly lesions were presented as unilocular radiolucent while few were multilocular. The expression of podoplanin was strongly positive in the basal and suprabasal layers of odontogenic keratocyst and surrounding inflammatory connective tissue stroma in inflamed cysts. In ameloblastoma, it was predominantly seen in the peripheral columnar cells of tumor islands, while the stellate-reticulum like cells showed weak or nearly no immunostaining.

Conclusion: The positive expression of podoplanin in odontogenic cysts and ameloblastoma suggests its role in proliferation and local invasive behavior.

Key Words: Podoplanin, Odontogenic cysts, Ameloblastoma

Introduction

Odontogenic cysts usually develop when odontogenic cell rests such as the epithelial remains of Malassez, the dental lamina (cell rests of Serres), or the enamel organ get entangled within the bone or gingival tissue of the jaws. However inflammatory odontogenic cysts are formed because of the stimulation of these cells rests by an inflammatory process [1].

Both developmental and inflammatory odontogenic cysts are epithelial in origin showing slow growth and expansion. Though they act biologically as benign these lesions can attain a marked increase in size if they are overlooked and not treated well on time [2].

Periapical or radicular cysts are the most common inflammatory odontogenic cystic usually found at the apices of the involved teeth [3]. They constitute about 52% to 68% of all the cysts affecting human jaws. Mostly they are symptomless and presented as slowly enlarging swellings [4].

Among the developmental odontogenic cysts, dentigerous cysts, also called follicular cysts, are the most common and frequent cystic lesions of the jaw after the radicular cysts. They report around 24% of all true cysts in the jaw [5]. These cysts are formed as a result of the expansion of dental follicles, because of fluid accumulation between the tooth crown and epithelial components [6].

Odontogenic keratocyst is also a developmental cyst first classified by Philipsen in 1956 and it develops from remnants of the dental lamina. It has a high growth rate thus showing an increased recurrence rate [7].

Ameloblastoma is the benign and one of the most common odontogenic tumors, locally aggressive with a high rate of recurrence. Histologically, it has different patterns; follicular, plexiform, acanthomatous, granular cell and basal cell variants. The mechanism of oncogenesis, cytodifferentiation, and tumor progression is still not known [8] but the advance studies propose the genetic and molecular changes in epithelial odontogenic tumors [9]. Regarding origin, odontogenic tumors tend to arise from the rest of Malassez, these cells exhibit distinctive properties of epithelial-mesenchymal interactions which are considered to be involved in different stages of tumor progression including invasion, resistance against apoptosis, and metastasis [10,11].

These odontogenic lesions can be harmful because of recurrence and aggressive growth as well as both benign and malignant transformation can occur in their in epithelial lining [12].

Radiographically odontogenic cysts and tumors can be seen as unilocular or multilocular radiolucent areas of different sizes, having smooth or scalloped periphery [13]. Moreover, tooth displacement, root resorption and impacted teeth can also be seen in relation to these cystic lesions [14]. These cystic and neoplastic lesions can be diagnosed on the basis of history, clinical, radiological and histopathological examination.

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Podoplanin is a 38 kDa type-1 transmembrane sialomucin-like glycoprotein thus compromising of 162 amino acids. Normally podoplanin is expressed in kidney podocytes, skeletal muscle, placenta, lung, heart myofibroblasts of the breast and salivary glands as well as in osteoblasts and mesothelial cells [15]. It is a lymphatic endothelial marker but its expected role in odontogenic tumorigenesis has just been found too. Its expression can be seen in odontogenic tissues like in secretory ameloblasts, developing and mature odontoblasts, tomes' fibers and pulp cells [16]. Podoplanin has also been seen to modulate the actin cytoskeleton that is suggestive of an essential role in tumor invasion and metastasis [15].

Literature shows that this protein reveals fairly a broad spectrum of reactivity in both benign and malignant oral tumors [17]. Different studies report the presence of podoplanin expression in various oral tissues and tumors, yet its physiologic or pathologic function is not much known. Sawa, et al. suggested an association of podoplanin with cellular proliferative activity due to its expression in cells with high mitotic activity [18].

Considering the above factors, this study was designed to observe the podoplanin expression in Dentigerous, Radicular, Odontogenic Keratocysts and Ameloblastoma in relation to invasive behavior and neoplastic potential of these lesions.

Materials and Methods

The present study was conducted in the Department of Morbid Anatomy and Histopathology University of Health Sciences after the approval from the Institutional Review Board Committee. A total of 44 cases were included in the present study, out of which 04 were of Dentigerous cysts, 11 Odontogenic keratocyte, 15 Radicular cysts and 14 were of Ameloblastoma. There were n=29 males and n=15 females. All the specimens were obtained from the blocks of the Department of Morbid Anatomy and Histopathology University of Health Sciences and Department of Oral Pathology Fatima Memorial Medical and Dental College Lahore. The study period was 8 months. The patient's age, gender, site, duration and size of the lesions were included in data. Hematoxylin and eosin-stained sections were prepared and looked by three histopathologists to confirm the diagnosis.

Immunohistochemistry

Approximately 04 microns sections were taken on poly L-lysine glazed glass slides. Deparaffinized tissue sections were dipped in 0.01 M citrate buffer, pH 6.0, and heated in a microwave oven for 5 min at high voltage followed by a low voltage for 15 min to retrieve antigen. The tissue was then placed in peroxide block for 20 min at room temperature to block endogenous peroxidase activity. Tissue was covered with power block reagent for 20 minutes once it was washed with phosphate-buffered saline. Ready to use mouse monoclonal anti-human D2-40 (anti-podoplanin) was applied to the sections for 1 hour at room temperature after that these were covered with Post-primary block for 25 minutes. Then sections were treated with secondary antibody conjugated with peroxidase. Then we applied freshly prepared substrate chromogen diaminobenzidine (DAB) solution for 2 minutes followed by counterstaining with Mayer's hematoxylin.

Immunostaining evaluation

Scoring was based on [19]:

The intensity of the podoplanin expression in the epithelial odontogenic and ameloblastic cells

(A): 0=absent, 1=weak, 2=moderate, 3=strong, and 4=very strong

Percentage of podoplanin positive odontogenic and ameloblastic 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)

Data were analyzed by using Pearson correlation, Mann-Whitney test and Kruskal-Wallis test. The level of significance was set at 5% for all tests.

Results

There were n=44 cases consisting of developmental cysts i.e dentigerous cyst (n=04), odontogenic keratocysts (n=11), inflammatory cysts (radicular cysts) (n=15) and ameloblastoma (n=14).

The age range was from 10 to 50 years (mean age =29.6 ± 13.05 years) and 15 to 42 years (mean age=27.2 ± 7.93 years) for the developmental and inflammatory odontogenic cysts (radicular cyst) respectively. Regarding gender distribution, there were n=10 (66.7%) males and 05 (33.3%) females (M:F; 2:1) in developmental odontogenic cysts while in radicular cysts there were 11 (73.3%) males and only 4 (26.7%) females. Most cases of developmental odontogenic cysts were seen in the posterior region of the mandible (86.7%) while comparing to the maxilla (13.3%). Similarly, n=10 (66.7%) cases of radicular cysts were seen in mandible and 5 (33.3%) in the maxilla. The size of developmental cysts was from 9 mm to 35 mm (mean size=22.1 ± 8.0255 mm) and 8 mm up to 21 mm (mean=14.6 ± 4.11) for the radicular cyst. The duration of swelling of both developmental and inflammatory odontogenic cysts was from 7 to 26 days and 2 to 14 days respectively.

In ameloblastoma, the age range was from 22 years to 54 years with a mean age of 42.5 ± 9.28 years. Most were males 8 (57.14%) and 6 (42.85%) were females. Almost all cases 13 (92.85%) were seen in the mandible and only one case (7.2%) has been found affecting the maxilla. A large variation was seen in size from 20 mm to 84 mm with a mean of 49.57 ± 16.26 mm and the duration of the lesion was from 15 days to 40 days.

Out of n=14 cases of ameloblastoma, the follicular pattern was found to be the most frequent 6 (42.85%) followed by unicystic n=4 (28.57%) and plexiform variety of ameloblastoma n=4 (28.57%).

Regarding gender distribution, male predominance was seen in odontogenic cysts and ameloblastoma. Statistically, no significant relation was seen between the age of the patients and gender (p=0.275; p>0.05). While the significant correlation was found between the age of the patient and size of lesion and duration of swelling (p<0.05) (*Table 1*)

Table 1. *Relation of patients' age with size and duration of swelling in odontogenic cysts and ameloblstoma.*

Variables		Mean	Std. Deviation	p-Value	Pearson Correlation	
Age	Size	28.29	18.23	0	0.658	p<0.05
	Duration	16.29	9.35	0	0.525	

A significant correlation was observed between the size of the lesion and duration of the lesion by using Pearson Correlation (r=0.870: p=0.000) while no positive relation was seen between the size and duration of lesion and staining intensity, proportion of positive cells and total scoring (p>0.05).

Clinically 25 of 44 (56.8%) patients presented with asymptomatic painless swelling, while n=10 (22.7%) presented with pain and n=9 (20.4%) were diagnosed incidentally on the radiograph as they came for other dental procedures (*Figure 1*).

Radiographically, 26 cases of odontogenic cysts (86.6%) n=34 cases were seen as well-defined unilocular radiolucencies and 4 cases (13.3) were shown up as unilocular radiolucent areas with scalloped borders. In ameloblastomas, 10 cases were seen as well corticated multilocular radiolucencies while 04 cases as unilocular radioluencies.

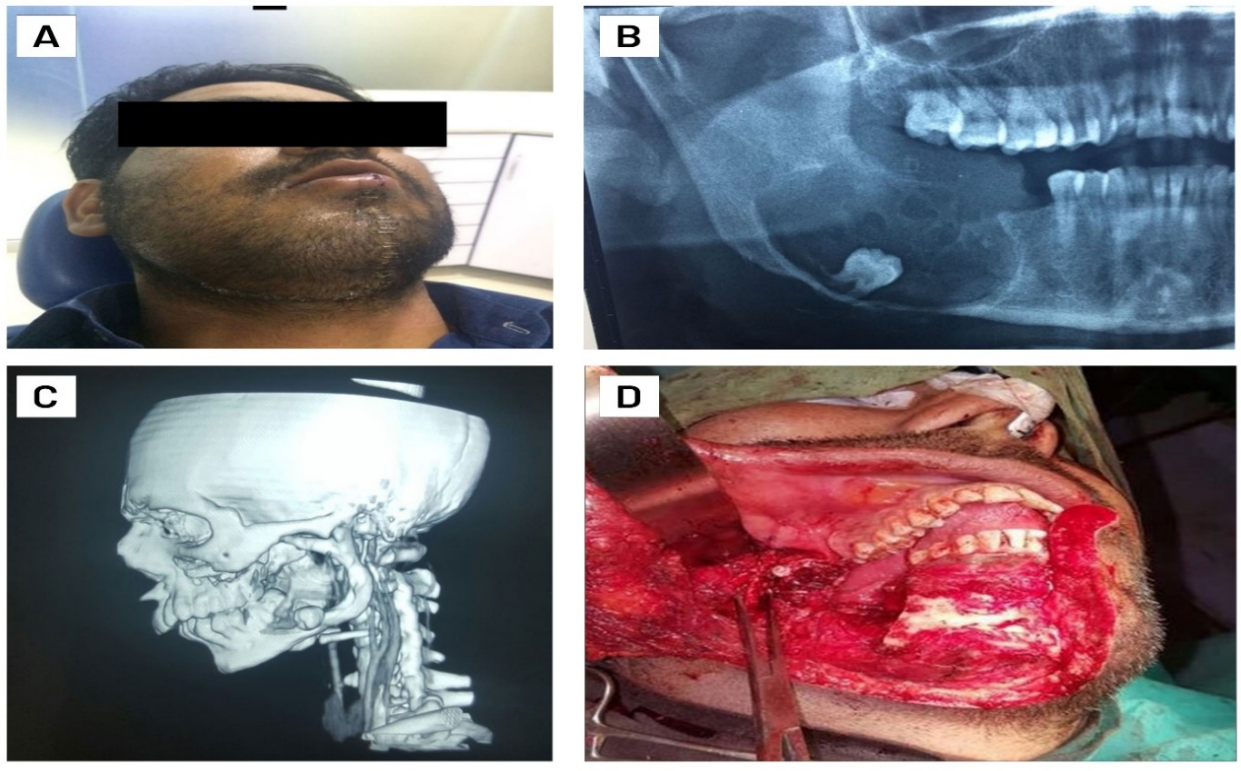

Figure 1. *(A) 32 Years old male presented with swelling on the right side of mandible; (B) Radiograph shows a large multilocular radiolucency around impacted tooth; (C) 3D CT scan showing the destructive part of mandible indicated by black arrows; (D) Showing surgical removal of affected part of mandible.*

The expression of podoplanin was strongly positive in the cell membrane and cytoplasm of most of the cells in the basal and suprabasal layers of odontogenic keratocyst (*Figure 2*). While in inflamed dentigerous cysts and radicular cyst positive immunoreactivity was seen only in cells of the basal layer and surrounding inflammatory connective tissue stroma (*Table 2*).

The strong expression of podoplanin in ameloblastomas was predominantly seen in the peripheral columnar cells of tumor islands, while the stellate-reticulum like cells showed weak or nearly no immunostaining.

Unicystic ameloblastomas showed variable podoplanin expression as it was limited to the basal and supra-basal layers of the cystic lining in these tumors (*Table 2*).

Statistically, no significant relation was found while the Mann-Whitney U test was used to compare the intensity of staining, the proportion of positive cells and total scores

among developmental and inflammatory odontogenic cysts (*Table 3*).

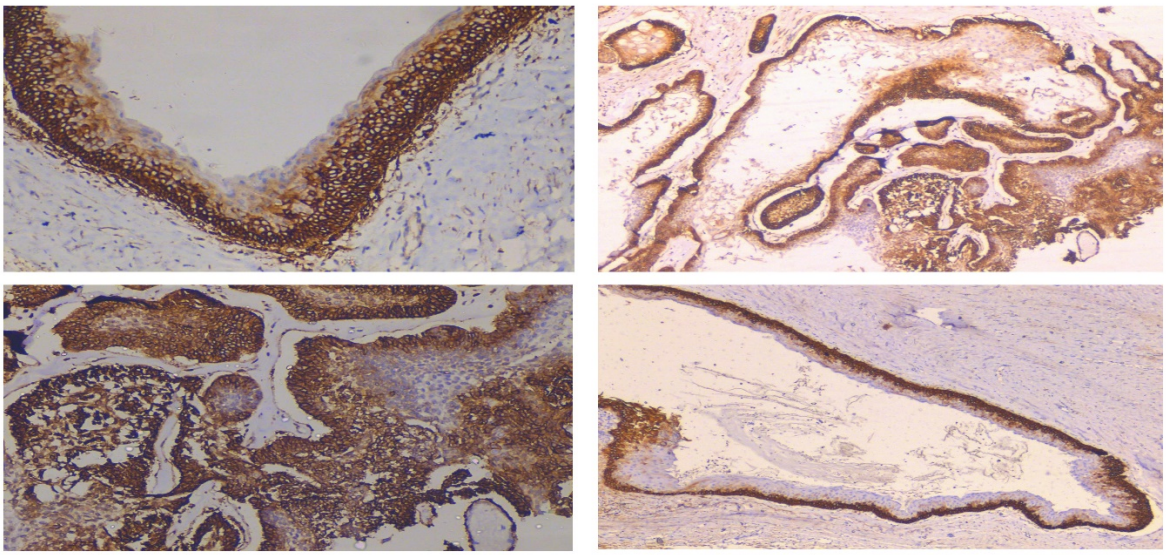

Figure 2. *This figure shows the positive podoplanin expression in odontogenic cysts and ameloblastoma.*

Table 2. *Frequency of podoplanin expression in odontogenic cysts and ameloblastoma.*

Odontogenic Cysts	N (%)	Podoplanin Expression		
		Absent (Score=0)	Weak (Score=1-4)	Strong (Score=5-8)
Developmental Cysts	15 (100)	Nil	2 (13.3)	13 (86.6)
Inflammatory Cyst	15 (100)	Nil	1 (6.6)	14 (93.3)
Ameloblastoma	14 (100)	Nil	5 (35.7)	9 (64.2)

Table 3. *This table shows the comparison of immunostaining in odontogenic developmental and inflammatory cysts.*

Group	The intensity of staining (A)		The proportion of positive cells (B)		Total Score (A+B)	
	Mann-Whitney U test	p-value	Mann-Whitney U test	p-value	Mann-Whitney U test	p-value
Developmental Odontogenic Cysts and Inflammatory Cysts	93	>0.05	88.5	>0.05	79.5	>0.05

Kruskal-Wallis H test χ^2 was applied to compare the intensity of staining, the proportion of positive cells and total scores among developmental, inflammatory odontogenic cysts and ameloblastoma and found no significant relation (*Table 4*).

Table 4. *This table shows a comparison of immunostaining in odontogenic cysts and ameloblastoma.*

Group	The intensity of staining (A)		The proportion of positive cells (B)		Total Score (A+B)	
	Kruskal-Wallis H test χ2 (2)	p-value	Kruskal-Wallis H test χ2 (2)	p-value	Kruskal-Wallis H test χ2 (2)	p-value
Developmental Odontogenic Cysts, Inflammatory Cysts and Ameloblastoma	2.608	0.271	2.445	0.295	4.424	0.109

Discussion

Odontogenic cysts and tumors make an unusual special group of lesions. In the present study, both developmental and inflammatory odontogenic cysts were reported equally 34.09% out of 44 cases [20]. Souza LB et al., found a higher frequency of inflammatory cysts in his study while Ledesma-Montes reported an increase frequency of developmental cysts. The reason behind this difference in the relative frequency of occurrence of inflammatory and developmental cysts can be the socioeconomic status of different populations [20].

Regarding odontogenic cysts, in the present study, the patients in the first to the fourth decade of life were the most commonly affected. While comparing to other studies which reported first to a fourth and 3^{th}-5^{th} decade of a life afflicted by odontogenic cysts respectively [21,22].

The most frequent anatomical location of odontogenic cysts was the mandibular posterior area in the present study which is very similar to other studies in which mandible to maxilla ratio was reported as 1.5 and 1.3:1 [23]. Concerning gender distribution, male predominance was seen in the present study which is in accordance with the finding observed by Johnson NR [21]. Different studies have been proposed that the greater occurrence in males could be related to poor oral hygiene and higher possibility of trauma than women as these both factors can lead to cystic lesion formation [24].

The literature revealed dentigerous and odontogenic keratocysts as radiolucent lesions with well-defined corticated boarder similar findings were observed in the present study. Dentigerous cysts are usually seen as unilocular, well-defined corticated radiolucent areas however they may be ill-defined due to infection or scalloped in big cysts [25]. Tsukamoto G reported 84% of cases seen as radiolucent lesions with smooth borders that are most likely to the present study [26].

Ameloblastomas are inexplicable oral tumors which usually have benign growth pattern but can invade locally and infrequently metastasize. They exhibit slow and persistent growth, disturbing the marrow spaces with pseudopods, without causing trabecular bone resorption [27].

With respect to age range in ameloblastoma, the age at the time of diagnosis was 44.1 years with a peak incidence between the second and sixth decade reported by Oomens in his study which is nearly similar to 42.5 ± 9.28 years observed in present [28]. In another study, Fregnani found the average age for ameloblastoma as 33.2 years among 121 cases from Brazil [29].

Tatapudi R observed 50% of the ameloblastomas in the body and posterior regions of the mandible in his study [30]. Literature also revealed that ameloblastomas more commonly affect the mandible, predominantly the posterior region which is in accordance with the present study [31]. Radiographically, More C reported all cases of his study as well defined radiolucent lesions in which mostly were multicystic and few were unicystic. Similar observations were seen in the present study.

Tatapudi R in his study reported unicystic ameloblastoma the most often encountered histological subtype (49.9%), followed by plexiform (20%), and follicular (10%), among the histopathological types [30]. Likewise, Pereira FD et al., in his study showed that unicystic variant of ameloblastoma is the most common histological subtype (34%), followed by follicular (19.8%), and plexiform (22%) [31]. While in the present study, follicular variant of ameloblastoma was found mostly.

In the present study, positive podoplanin expression was seen in the only basal layer of epithelium and surrounding inflamed connective tissue of dentigerous cyst and periapical (radicular cyst). Similar findings were observed by Okamoto et al. who conducted his study to observe the correlation of podoplanin expression with a neoplastic feature and a significant factor for assessing odontogenic tumors [16]. He reported the strong positivity for podoplanin in the basal layers of dentigerous cyst associated with a severe inflammatory reaction in the connective tissue while few cases of dentigerous cysts without an inflammatory reaction were completely negative for podoplanin in the cells of basal layer [16].

Tijoe KC et al. also reported positive immunoexpression of podoplanin in the inflamed radicular cyst. Similar findings were observed in the present study and by other authors [32]. Although inflammatory odontogenic cysts develop from inflammation, while its role in the formation of developmental cysts is still not established [33]. But all these odontogenic cysts have a similar odontogenic epithelial origin thus showing variable biological behaviors as well as invasion patterns [19].

Namrata Singhal et al. also reported positive immunoexpression of podoplanin in the basal layer only in inflamed dentigerous cyst while it was negative or weak in some areas of lining epithelium. He did his study to analyse the expression of podoplanin in the ameloblastomas and odontogenic keratocyst to explain and support the significance of this molecule in the growth of these tumors and comparing their expression pattern with dentigerous cysts [19].

The present study reported the strong podoplanin expression in basal and parabasal layers of odontogenic keratocysts which are similar to findings observed by Namrata Singhal et al. in his study. Thereby it suggesting the proliferative activity of these cells as well as escalating their potential for intrinsic growth and also making them destructive and invasive locally. That's why it is believed that podoplanin together with other proteins and growth factors play an important part in accelerating the proliferative activity of the lining epithelium in odontogenic keratocyst [19].

Likewise, Sarmiento VL et al., also reported the strong podoplanin expression in basal and parabasal layers of odontogenic keratocysts [22]. The pattern of staining for podoplanin in odontogenic keratocyst could be related to its neoplastic nature and may suggest a role of this protein in its invasiveness [6]. Both mitotic activity and podoplanin expression within the ameloblastoma limited to the peripheral epithelial cells of the tumor cords and strands are co-existed. This pattern of distribution of podoplanin immunostaining in relation to cellular subtype in ameloblastomas may be helpful in classifying odontogenic tumors [34].

Ganvir SM who conducted his study to observe the podoplanin expression in ameloblastomas to determine its role in progression and invasion of tumors reported the membranous and cytoplasmic expression of podoplanin in epithelial tumoral cells while the central cells of the islands were negative [34]. These findings were corroborated with the findings observed by González-Alva et al. [17] and Zustin et al. [35] in their studies. Similar findings were seen in the present study. The plexiform variant of ameloblastoma bears a resemblance to the tooth germ in the dental lamina stage of tooth formation when the differentiation process of the odontogenic epithelium has not started yet. This lack of cellular differentiation may affect podoplanin expression in benign epithelial tumors and similar findings were observed by other authors [36].

The present study showed no significant relation between staining intensity, the proportion of positive cells and total scoring while comparing odontogenic cysts and ameloblastoma. Similarly, Namrata Singhal et al. found no significant comparison except between ameloblastoma and dentigerous cysts [19]. He also found a significant relation between dentigerous cysts and radicular cysts while there was no significant relation between dentigerous cysts and odontogenic keratocysts as seen in the present study. These data favors the impression that podoplanin may be important for running the proliferative activity of the epithelial odontogenic cells. While few authors proposed that podoplanin may be linked with cellular proliferative activity depending on its expression in odontogenesis [27]. Remarkably, a similar pattern of podoplanin expression seen in the odontogenic epithelium of murine developing teeth where pre-ameloblasts strongly express podoplanin while differentiating into mature ameloblasts, decreasing podoplanin immunoreactivity.

Conclusion

The positive expression of podoplanin in the odontogenic epithelium of odontogenic keratocyst and ameloblastomas signify that it affects the proliferative activity of these cells and accelerates their intrinsic growth potential. Therefore podoplanin can be used as a potential proliferative marker to observe the biological behavior of dentigerous cyst and radicular cyst. Also, the staining pattern of podoplanin in odontogenic keratocyst and ameloblastoma could be related to their aggressive and invasive behaviors. It also recommends that podoplanin expression is essential for the processes having high cellular activities such as proliferation and differentiation. Thus this protein looks to contribute in the process of local invasion of odontogenic keratocyst and neoplasia like ameloblastoma by composing the cytoskeleton movement. However further studies can be done to comprehend the actual molecular mechanisms of podoplanin that show its involvement in these odontogenic lesions by studying a larger sample size.

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