UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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EXPRESSÃO DE PODOPLANINA EM CERATOCISTOS ODONTOGÊNICOS ASSOCIADOS OU NÃO ASSOCIADOS À SÍNDROME DO CARCINOMA NEVOIDE DE CÉLULAS BASAIS

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Trabalho de Conclusão de Curso apresentado a Curso de Graduação em Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Cirurgiã-Dentista.

Orientadora: Profª. Drª. Márcia Gaiger de Oliveira.

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Porto Alegre, 9 de julho de 2019

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À minha mãe por sonhar comigo e doar todo seu esforço e dedicação de forma incondicional para que eu pudesse percorrer um caminho confortável e sólido. Obrigada por ser um exemplo de força e resiliência frente as dificuldades, reforçando sempre que com trabalho, paciência e dedicação alcançamos nossos sonhos.

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Ao meu companheiro Gustavo por ser um parceiro nessa longa jornada, vivenciando cada etapa ao meu lado, me apoiando e compartilhando todo seu afeto, cuidado, leveza e respeito, características predominantes da sua essência que são fundamentais na minha profissão.

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RESUMO

Resumo: a podoplanina é uma proteína transmembrana expressa em várias células normais ou neoplásicas. Alguns estudos têm mostrado que a podoplanina promove a migração e a invasão de células tumorais. Esse estudo avaliou a expressão de podoplanina em Ceratocistos Odontogênicos (CO) associados ou não associados à Síndrome do Carcinoma Nevóide de Células Basais (SCNCB) e Cistos Odontogênicos Ortoceratinizados (COO). Materiais e Métodos: um total de 50 lesões foram obtidas nesse estudo, 28 COs, 18 COs associados à SCNCB e 4 COOs. A expressão imunoistoquímica da podoplanina em células epiteliais da camada basal e suprabasal foi avaliada usando os seguintes escores: (a) intensidade da imunomarcação (0: ausente, 1: fraco, 2: moderado, 3: forte e 4: muito forte) e (b) número de células positivas (0: 0%, 1 = <25%, 2 = 25% a 50%, 3 = 50% a 75% e 4 = >75%). O escore final foi determinado pela soma dos escores, variando de 0 a 8 (0: ausente, 1-4: fraco e 5-8: forte). Resultados: a expressão de podoplanina foi significativamente mais forte na camada basal das lesões de COs e das associadas à SCNCB. Além disso, a expressão de podoplanina foi maior na camada suprabasal de lesões da SCNCB, seguido pela suprabasal de COs e de COOs. Conclusão: a expressão de podoplanina é diferente em lesões de diferentes comportamentos biológicos. O padrão e a localização da expressão da podoplanina poderiam sugerir a sua influência em atividades de proliferação e migração celular.

Palavras-chave: Ceratocisto odontogênico. Síndrome do carcinoma nevóide de células basais. Cisto odontogênico ortoceratinizado. Podoplanina.

ABSTRACT

Background: Podoplanin is a transmembrane glycoprotein expressed on various normal or neoplastic cells. Some studies have shown that podoplanin promotes the migration and invasion of tumor cells. This study evaluated podoplanin expression in Odontogenic Keratocysts (OKs) associated or not associated with Nevoid Basal Cell Carcinoma Syndrome (NBCCS) and in Orthokeratinized Odontogenic Cysts (OOCs). Materials and Methods: A total of 50 lesions were obtained in this study, 28 OKs, 18 OKs associated with NBCCS, and 4 OOCs. Immunohistochemical expression of podoplanin in epithelial cells was evaluated using the following score: (a) intensity of immunostaining: (0: absent, 1: weak, 2: moderate, 3: strong, and 4: very strong) and (b) number of positively cells (0 = 0%, 1 = <25%, 2 = 25% to 50%, 3 = 50% to 75% and 4 = >75%). The final score was determined by adding the scores ranging from 0 to 8 (0: absent, 1 to 4: weak, and 5 to 8: strong). Results: Podoplanin expression was significantly stronger in the basal layer OKs and NBCCS lesions. Further, podoplanin expression was the highest in the suprabasal layer of NBCCS lesions, followed by the suprabasal layers of OK and OOC lesions. Conclusion: Podoplanin expression is different in lesions of different biological behaviors. The pattern and localization of podoplanin expression may suggest its influence on cell proliferation and migration.

Keywords: Odontogenic keratocyst. Nevoid basal cell carcinoma syndrome. Orthokeratinized odontogenic cyst. Podoplanina.

SUMÁRIO

1 INTRODUÇÃO

1.1 CERATOCISTOS ODONTOGÊNICOS

Os ceratocistos odontogênicos (CO) são lesões intra-ósseas, uni ou multicísticas que tem origem da lâmina dentária e seus remanescentes[1]. Clinicamente acomete indivíduos desde a primeira até a nona década de vida com maior frequência na segunda e terceira década e com discreta predileção para o sexo masculino. O sítio de maior acometimento é a mandíbula (65 a 83% dos casos), principalmente na região de ângulo, estendendo-se ântero-posteriormente, com crescimento medular, causando pouca expansão das corticais. Imaginologicamente observa-se lesão radiolúcida bem delimitada com margens escleróticas[1]

Microscopicamente são formados por epitélio pavimentoso estratificado paraceratinizado com superfície corrugada, espessura de 5 a 8 camadas com interface epitélio conjuntivo plana e camada basal bem definida, no geral, com células cúbicas ou colunares hipercromáticas e em paliçada, com núcleos localizados distantes da membrana basal. Esse epitélio aparece em algumas regiões destacado da fina cápsula de tecido conjuntivo. Pode-se observar também freqüentemente a presença de cistos satélites ou cistos filhos na cápsula[1,2].

Em 2005, a Organização Mundial de Saúde (OMS) classificou-os como um neoplasma benigno, renomeando para tumor odontogênico devido ao comportamento clínico agressivo, a alta taxa de recorrência, a associação com a Síndrome do Carcinoma Nevóide de Células Basais (SCNCB) e a presença de mutações nos genes supressor de tumor PTCH[3,4]. Em 2017, a OMS reverteu a terminologia para cistos de desenvolvimento odontogênicos porque muitos artigos mostraram que a mutação no gene PTCH poderiam ser encontrados em lesões não neoplásicas, incluindo cistos dentígeros, e porque a resolução do cisto após marsupialização não seria compatível com um processo neoplásico[4-6].

O ceratocisto odontogênico pode apresentar-se de forma solitária ou múltipla, sendo essa última muitas vezes relacionada com a Síndrome do Carcinoma Nevóide de Células Basais ou Síndrome de Gorlin-Goltz[7]. Essa síndrome é hereditária, autossômica dominante, com alta penetrância, porém pode manifestar-se espontaneamente e a sua expressividade é variável, sendo causada por mutações também no gene PTCH. A inativação do supressor tumoral ocorre por mutação com

perda de um homólogo por não-disjunção, deleção ou recombinação[8]. As principais características dessa síndrome são múltiplos carcinomas de células basais em pele, múltiplos ceratocistos odontogênicos e anomalias crânio-esqueléticas.

Os múltiplos ceratocistos odontogênicos ocorrem em 65 a 75% dos pacientes, geralmente em área de ângulo e ramo de mandíbula e túber, sendo uma das primeiras manifestações da síndrome na primeira década de vida. Os carcinomas nevóides basocelulares cutâneos ocorrem em 80% dos pacientes brancos e em 35% dos negros. As alterações esqueléticas são variadas e as mais freqüentes são: costela bífida, escoliose, fusão de vértebras, espinha cervicotoráxica bífida, calcificações cranianas, prognatismo mandibular, bossas frontais e temporoparietais, encurtamento de metacarpos e hipertelorismo. São comuns também sinais cutâneos palmares e plantares[8-10].

Essas duas variedades de ceratocisto odontogênico (isolado ou associado à Síndrome) não apresentam o mesmo comportamento biológico, velocidade de crescimento e taxa de recidiva, segundo alguns autores[11-14]. A multiplicidade de cistos, o número maior de cistos satélites e ilhas epiteliais nos cistos associados à síndrome sugerem um comportamento mais agressivo e pode estar associado com maior atividade proliferativa epitelial[11, 15]. Além disso, observaram-se maiores taxas de proliferação celular e expressão de oncoproteínas e genes supressores de tumor como o PCNA[16,17], Ki-67[15-17] e p53[16,18,19].

Existe ainda uma variante dessa lesão cística onde o epitélio é ortoceratinizado, sendo menos agressivo. Essa lesão é chamada de cisto odontogênico ortoceratinizado[20]. Essa denominação refere-se a um cisto odontogênico que microscopicamente apresenta limitante epitelial ortoceratinizado. Clinicamente difere do ceratocisto odontogênico, constituindo de 7 a 17% dos cistos ceratinizados dos maxilares. Essa lesão predomina em adultos jovens, com relação homem-mulher de 2:1. Ocorrem duas vezes mais na mandíbula, com tendência a envolver regiões posteriores. Imaginologicamente apresenta-se como lesão radiolúcida unilocular bem delimitada, podendo ocasionalmente ser multilocular. Essa variante não está associada à síndrome do carcinoma nevóide de células basais. Microscopicamente é constituído de epitélio escamoso estratificado, com superfície ortoceratinizada de espessura variável e não há uma camada basal proeminente em paliçada característica, como no ceratocisto odontogênico. Seu comportamento é bem menos agressivo, com taxa de recorrência baixa, por volta de 2%[15,20,21].

1.2 PODOPLANINA

A podoplanina é uma glicoproteína transmembrana tipo I que apresenta a estrutura típica de uma proteína deste tipo, contendo um domínio extracelular hidrofílico, um transcelular hidrofóbico e uma curta cauda citoplasmática constituída por 9 aminoácidos[22]. Acredita-se que essa porção interna da podoplanina seja responsável por se ligar às proteínas da família ERM (ezrin, radixin e moesin)[23].

Foi reportada sua expressão em células endoteliais do plexo coroide cerebral, células alveolares tipo I do pulmão, células mesoteliais do peritônio, osteoblastos e osteócitos. Subsequentemente esta proteína foi identificada nos podócitos, células epiteliais glomerulares de ratos, fato que inspirou seu nome, hoje consagrado[24]. Por não ser expressa no endotélio de vasos sanguíneos, a podoplanina tem sido utilizada largamente como marcador de vasos linfáticos[26,27]. O anticorpo anti-podoplanina mais utilizado é o D2-40[26].

A podoplanina tem intrigado pesquisadores devido a sua variedade de expressão que vai desde as estruturas supracitadas até miofibroblastos de glândulas salivares de ratos[28], mama e próstata, células foliculares dendríticas e condrócitos[23,26,29.] Células epiteliais de revestimento da mucosa bucal também podem expressar a proteína[29,30].

Além disso, a podoplanina é expressa numa variedade de neoplasias como o carcinona espinocelular de cabeça e pescoço (incluindo o bucal), pele, esôfago, pulmão, câncer de ovário, testículo, cérebro, mesotélio, cérebro e trato gastro-intestinal[26,31].

No carcinoma espinocelular de boca, a expressão da podoplanina tem sido relatada em áreas de displasia ou de hiperplasias adjacentes a neoplasia[26]. A podoplanina se expressa em dois padrões: distribuição difusa pelas células tumorais ou confinada ao front de invasão tumoral, e também tem sido associada à metástase de linfonodos[27,31].

A transição epitelial-mesenquimal (TEM) é um processo pelo qual uma célula

epitelial deixa de expressar marcadores epiteliais e passa a expressar marcadores mesenquimais[23], ocorrendo alterações fenotípicas e funcionais, como mudança na forma da célula epitelial, que passa a adquirir aspecto fibroblástico, com emissão de projeções citoplasmáticas e maior motilidade, garantindo interação com outras células

e tendo capacidade de invasão aos tecidos adjacentes[23,24]. Foi demonstrado que a podoplanina participa da TEM em células MDCK ao se ligar a ezrin, membro da família de proteínas ERM (ezrin, myosin e radixin), através da sua cauda citoplasmática[23]. Esta interação ativa a GTPase RhoA, proteína envolvida em diversas atividades celulares, entre elas a proliferação, diferenciação, transformação e motilidade celular[31]. Através da ativação da GTPase RhoA se dá inicio a remodelação do citoesqueleto de actina e formação de projeções citoplasmáticas. Assim, a célula adquire acentuada capacidade de invasão tecidual[23].

A associação da podoplanina com a TEM, no entanto, ainda é tema de controvérsia. Wicki et al[33] demonstraram que a invasão e migração celular induzida pela podoplanina pode ocorrer mesmo na ausência da TEM em cultura de células de câncer de mama.

Assim, duas hipóteses para a função da podoplanina foram levantadas. A primeira leva em consideração o fato de a proteína ser expressa na porção terminal da bainha epitelial de Hertwig durante a formação da coroa dentária[34] e na camada basal do epitélio bucal hiperplásico[35], localizações com grande proliferação celulares. Suporta ainda esta hipótese o fato da expressão da podoplanina e do Ki-67 coincidirem nos germes dentários em odontogênese[34,36]. Soma-se a isto o fato de a podoplanina ser expressa no front de invasão tumoral de vários tumores malignos e benignos citados acima, como carcinomas espinocelulares e ameloblastomas[27,37]. Ademais, estudos in vitro têm sugerido que a podoplanina está associada a atividade de migração e/ou remodelação celular, processos inerentes a mitose[22,23,27,33].

A segunda hipótese é a de que a podoplanina esteja associada a TEM ao participar da remodelação do citoesqueleto celular tanto em processos fisiológicos como de invasão tumoral[23,38]. Isso porque células com menor grau de diferenciação expressam-na, enquanto as que tem maior grau de maturação não[38]. Em tumores odontogênicos, somente a segunda hipótese foi testada através da aferição da relação da expressão da podoplanina com a da vimentina (marcador mesenquimal) e da E-caderina (marcador epitelial) em ameloblastomas.

1.3 PODOPLANINA EM TECIDOS E TUMORES ODONTOGÊNICOS

O estudo da expressão da podoplanina em tecidos e tumores odontogênicos é um tópico relativamente recente. Sawa et al[34] em um estudo com germes dentários

observaram que na fase de pré- ameloblastos as células expressam fortemente a podoplanina, mas a partir do momento em que se diferenciam, os ameloblastos, curiosamente, deixam de expressá-las. As células epiteliais da alça cervical que apresentam alto índice mitótico expressam fortemente a podoplanina, enquanto as células frouxamente dispostas do retículo estrelado e do estrato intermediário fracamente sensibilizam-se. Já os odontoblastos reagiram intensamente ao anticorpo anti- podoplanina em todas as fases de diferenciação, concluindo-se que a podoplanina exerce possivelmente papel fundamental na diferenciação de células do germe dentário.

Gonzales-Alva et al[38] investigaram a expressão da podoplanina em ameloblastomas para avaliar seu papel na progressão tumoral, utilizando também marcador epitelial (E-caderina) e mesenquimal (vimentina) para averiguar se a TEM ocorre em ameloblastomas e qual sua associação com a podoplanina. A expressão da podoplanina que ocorreu em quase todos os casos com distribuição variável, predominantemente observada nas células periféricas do tumor, enquanto as semelhantes ao retículo estrelado foram negativas ou expressaram-se fracamente. A E-caderina apresentou imunoreatividade nas células centrais e bem diminuída nas periféricas, não havendo marcação para a vimentina. Não houve associação entre os marcadores e os autores concluíram que a podoplanina está associada aos tecidos neoplásicos de origem odontogênica e pode desempenhar papel na migração celular coletiva das ilhotas de ameloblastoma, possivelmente por meio da remodelação do citoesqueleto, porém sem relação com a TEM.

Okamoto et al[37] estudaram a expressão da podoplanina em tumores odontogênicos ceratocísticos e cistos odontogênicos ortoceratinizados. A grande maioria dos tumores odontogênicos ceratocísticos expressaram fortemente a podoplanina enquanto que quase todos os cistos ortoceratinizados não apresentaram reação. A imunoreatividade era predominante nas camadas basal e suprabasal do epitélio, sendo sugerida uma possível contribuição da podoplanina na invasão local do tumor odontogênico ceratocistico e associação com sua natureza neoplásica. Caetano et al[39] também obtiveram resultados semelhantes analisando[20] ceratocistos odontogênicos e 5 cistos odontogêncios ortoceratinizados. No entanto não se diferenciou na amostra a presença ou não da Sindrome do Carcinoma Nevóide de Células Basais.

2 OBJETIVOS

2.1 OBJETIVOS GERAIS

O propósito do nosso estudo foi comparar a expressão imunoistoquímica da podoplanina em CO isolado, em CO associados à SCNCB e em COOs, lesões de mesma origem, porém de comportamentos clínicos e biológicos distintos.

2.2 OBJETIVOS ESPECÍFICOS

a) avaliar as propriedades invasivas dos CO isolados, associados à SCNCB e de COOs e a sua relação com a expressão imunoistoquímica da podoplanina;

b) avaliar a expressão da podoplanina nos casos de ceratocistos odontogênicos diagnosticados no Laboratório de Patologia bucal da Universidade Federal de Santa Catarina e da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, no período de 1970 a 2013 e compará-los com outros estudos.

3 ARTIGO CIENTÍFICO

O desenvolvimento do trabalho está apresentado na forma de artigo científico de periódico inglês, aceito para publicação em 15 de maio de 2019 pelo periódico Applied Immunohistochemistry & Molecular Morphology.
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Podoplanin Expression in Odontogenic Keratocysts Associated or not Associated With Nevoid Basal Cell Carcinoma Syndrome.

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Background: Podoplanin is a transmembrane glycoprotein expressed on various normal or neoplastic cells. Some studies have shown that podoplanin promotes the migration and invasion of tumor cells. This study evaluated podoplanin expression in Odontogenic Keratocysts (OKs) associated or not associated with Nevoid Basal Cell Carcinoma Syndrome (NBCCS) and in Orthokeratinized Odontogenic Cysts (OOCs).
Material and Methods: A total of 50 lesions were obtained in this study, 28 OKs, 18 OKs associated with NBCCS, and 4 OOCs. Immunohistochemical expression of podoplanin in epithelial cells was evaluated using the following score: (a) intensity of immunostaining: (0: absent, 1: weak, 2: moderate, 3: strong, and 4: very strong) and (b) number of positively cells (0 = 0%, 1 = <25%, 2 = 25% to 50%, 3 = 50% to 75%

and 4 = >75%). The final score was determined by adding the scores of a and b and ranged from 0 to 8 (0: absent, 1 to 4: weak, and 5 to 8: strong).

Results: Podoplanin expression was significantly stronger in the basal layer OKs and NBCCS lesions. Further, podoplanin expression was the highest in the suprabasal layer of NBCCS lesions, followed by the suprabasal layers of OK and OOC lesions.

Conclusion: Podoplanin expression is different in lesions of different biological behaviors. Podoplanin seems to play a role in cell proliferation and migration.

Key Words: odontogenic keratocyst, nevoid basal cell carcinoma syndrome, orthokeratinized odontogenic cyst, podoplanin

INTRODUCTION

Odontogenic keratocysts (OKs) are benign odontogenic intraosseous lesions originating from the odontogenic epithelium. They may be present as a single lesion or as multiple lesions and are mainly located in the mandible. Multiple OK lesions may be associated to nevoid basal cell carcinoma syndrome (NBCCS).

NBCCS, also referred to as Gorlin–Goltz syndrome, is a rare autosomal dominant condition caused by mutations in the patched gene (*PTCH*), a tumor suppressor gene located on chromosome 9q22-q31, and has an incidence of approximately 1 in every 19,000 individuals, without any predilection for sex. NBCCS is characterized by multiple basal cell carcinomas, multiple OKs, bifid ribs, falx cerebri calcification, palmar and plantar pits,etc. Maxillary cysts are the most constant aspect of the syndrome, being present in at least 75% of the patients[1]. Often the cysts are multiple, appearing both in mandible and in maxilla, and affect younger patients than in those with isolated OK. Rates of NBCCS recurrence range from 0% to 50% depending on the treatment administered[1-7].

Orthokeratinized odontogenic cysts (OOCs) are clinically, radiographically and microscopically very similar to OKs and have been considered a less-aggressive variant of OKs. OOCs show typical clinical features of cystic lesion, i.e., water exchange between proliferating cavity and capsule. The recurrence rate of OOCs is very low, and they do not induce NBCCS [1,7-8].

PODOPLANIN

Podoplanin is a transmembrane glycoprotein commonly used as a marker for lymphatic vessels and expressed on various normal or neoplastic cells. Recent studies

have shown the relationship of this protein with cellular proliferative activities and cell expansion, migration and invasion in different types of lesions both in vivo and in vitro [8-15].

Studies assessing different odontogenic lesions suggest that podoplanin plays a role in cell migration, cell division, and tumor invasion possibly through cytoskeletal remodeling [9, 11,16-18]. A portion of podoplanin interacts with ezrin, which is a part of ezrin, radixin, and moesin complex, and promotes actin cytoskeleton reorganization and cell mobility through filose pseudopods, indicating that podoplanin promotes cell migration and metastasis[11,16,19].

Podoplanin is mainly expressed by peripheral cells (invasion front) of odontogenic tumors and appears to increase the risk of malignant transformation in some oral lesions. Moreover, podoplanin expression is stronger in aggressive ameloblastomas than in less-aggressive ameloblastomas, therefore it has been suggested to be a potential biomarker to observe the behavior of the lesions and to predict the risk of cancer in premalignant lesions[9, 11,12, 16, 17, 20-26].

In 2018, Li Y. et al, studied the tumor-stroma interaction in vitro in a 3D co-culture system with oral squamous cell carcinoma cells (OSCC) and fibroblasts and observed that podoplanin-positive OSCC cells create a basis for invasion and activation of fibroblasts[27].

Among the different odontogenic cysts, podoplanin expression is high in OKs and is almost absent in OOCs. Podoplanin expression is high in epithelial cells present in the basal and suprabasal layers of OKs and is suggested to contribute to OK invasion[11, 16,17,24]. In 2013, Caetano et al[11] observed a strong correlation between the expression of podoplanin and mitotic rate of epithelial cells in OKs and a weak correlation between the expression of podoplanin and index of cell proliferation in OOCs[11]. In addition, podoplanin expression has been detected in peripheral tumor cells in the basal epithelial layer and in regions showing high cellular activity.

The purpose of our study was to compare the immunohistochemical expression of podoplanin in OKs associated or not associated with NBCCS and in OOCs and to evaluate the expression in the lesions of different biological behaviors.

MATERIAL AND METHODS

This study was approved by the Research Ethics Committee of Federal University of Rio Grande do Sul (UFRGS) and Federal University of Santa Catarina

(UFSC). (process: 16628213.1.1001.0121 and process:01359312.3.0000.5347). Surgical specimens of OKs, NBCCS, and OOCs analyzed in this study were obtained of archives of both institutions from 1970 to 2013.

The study included 28 patients with OKs, 18 patients with NBCCS and 4 patients with OOCs. Odontogenic cyst samples obtained from these patients were fixed in formalin and embedded in paraffin. Hematoxylin and eosin stained (HE) paraffin sections were assessed by two experienced oral pathologists. OKs and OOCs were diagnosed using clinical, radiographic, and microscopic criteria[28], and NBCCS was diagnosed using a specific protocol proposed by Visioli et al in 2010 [25].

Immunohistochemical Analysis

For this, 4-µm-thick cyst sections were deparaffinized and rehydrated using ethanol. Antigen retrieval was performed by treating the sections with 10 mM citrate buffer (pH 6.0) for 4 min, and endogenous peroxidase activity was blocked by incubating the sections in 3% $H_2O_2$for 20 min. Next, the sections were incubated overnight at 48°C with anti-podoplanin primary monoclonal antibody (D2-40 clone, code#3619-1; Dako North America, Inc., Carpinteria, CA, USA) diluted by 1:200 in phosphate-buffered saline (PBS) and with bovine serum albumin (cat. #A2153; Sigma-Aldrich, St Louis, MO, USA) to block any non-specific reaction.The EnVisionTM+ system (Dako, Carpinteria, CA, USA) and 3, 3-diamino benzidine (Dako, Carpinteria, CA, USA) were used for visualization. Next, samples were counterstained with Mayer's hematoxylin, dehydrated, and covered with a cover slip. Oral lymphangioma sections were used as positive control, and lymphatic vessels of the studied lesions were used as internal positive control. For a negative control, the primary antibody was omitted during the immunohistochemical staining.

Immunohistochemical Scoring

As described previously[12,13,16,29] score of podoplanin expression in the odontogenic epithelium was determined by adding the intensity of immunostaining (0: absent; 1: weak; 2: moderate; 3: strong and 4: very strong) and percentage of positively stained (0: no staining; 1: <25%; 2: 25%-50%; 3 = 51%-75% and 4: >75%). Final score was determined as absent, weak and strong when the final results were 0, 1-4 and 5-8, respectively. This evaluation was done in basal and suprabasal epithelial layers.

Statistical Analysis

Kolmogorov–Smirnov test was used to confirm sample distribution. Kruskal–Wallis and Wilcoxon tests were used to determine the difference in podoplanin expression among the different lesions. The level of significance was set at 5% for all the tests.

RESULTS

Clinical Features

A total of 50 lesions were obtained in this study, including 28 OKs, 18 NBCCS and 4 OOCs. Of these, 25 (50%) were women and 25 (50%) were men, with an age range of 4–79 years (mean, 32.2 years). Moreover, most patients (90%) included in the study were white. In most patients, the odontogenic lesions were located in the posterior region of the mandible (52%), followed by the posterior region of the maxilla (18%) and the anterior and posterior regions of the mandible (14%).

Podoplanin Expression

Podoplanin expression was detected in all the lesions. Moreover, podoplanin expression was detected in the basal layer of the normal oral mucosa, lymphatic vessels, and odontogenic epithelial cells of the lesions, thus confirming the integrity of the immunohistochemical reaction. In OK and NBCCS lesions, strong podoplanin expression was detected in the basal layer and weak podoplanin expression was detected in the suprabasal layer (Fig. 1A and 1B). In OOC lesions, weak podoplanin expression was detected in both the basal and suprabasal epithelial layers (Fig. 1C).

The intensity, percentage, and final score of podoplanin expression were significantly high for the basal layer of OK lesions, followed by those for the suprabasal layers of NBCCS and OOC lesions. Moreover, the intensity and final score of podoplanin expression were high for the suprabasal layer of NBCCS lesions, followed by those for the suprabasal layer of OK and OCC lesions.

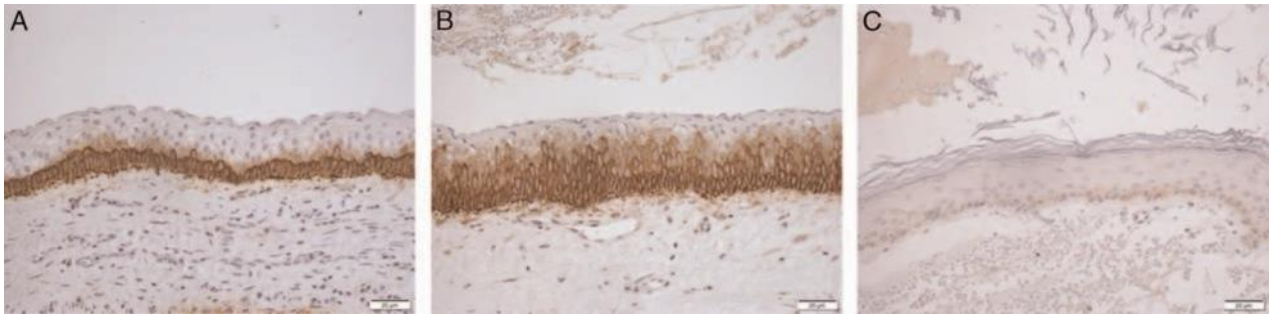

FIGURE 1. Immunoistochemical expression for podoplanin odontogenic keratocyst (A) and nevoid basal cell carcinoma syndrome (B) showing strong podoplanin expression in the basal layer and weak podoplanin expression in the suprabasal layer. Orthoker- atinized odontogenic cyst (C) showing weak podoplanin expression in both basal and suprabasal layers.

No differences were observed between OK and NBCCS lesions with respect to the percentage and final score of podoplanin expression; however, the percentage and final score of podoplanin expression were higher for OK and NBCCS lesions than for OCC lesions. These data are summarized on Table 1.

TABLE 1. Podoplanin immunoexpression by OKs, OKs associated with nevoid basal cell carcinoma syndrome and orthokeratinized odontogenic cysts.

	Basal Layer, n (%)			Suprabasal Layer, n(%)		
	OK	**Syndrome**	**OOC**	**OK**	**Syndrome**	**OOC**
Absent	1(3,6)	0 (0)	0 (0)	1 (3.6)	0 (0)	0
Weak	0 (0)	1 (5,5)	3 (75)	23 (82.1)	12(66.6)	4(100)
Strong	27(96,4)	17 (94,4)	1 (25)	4 (14.3)	6 (33.3)	0
Total	28 (100)	18 (100)	4 (100)	28 (100)	18(100)	4(100)
P	0.0033	0,001	0,0004	0.0126	0.0001	0.0015

Kruskal-Wallis test; statistical significant at p<0.05%
OK indicates odontogenic keratocyst; OOC – orthokeratinized odontogenic cyst, Syndrome, OK associated with nevoid basal cell carcinoma

DISCUSSION

Clinical characteristics of the 50 patients included in our study, such as sex, age, ethnicity, and lesion location, are consistent with those reported previously[28].

OOC are rare lesions, representing approximately 0.4% of the incidence of all odontogenic cysts, which justifies the low number of the sample in this study [1,30]. Nevertheless, it was possible to observe that the expression of podoplanin in the basal layer of these lesions was weak when compared to OK and NBCCS. This result can be explained by the different biological and clinical behavior among the lesions studied[1]. OK lesions show high rate of recurrence and proliferative activity and aggressive local behavior. The incomplete surgical removal of the epithelial

components contributes to recurrence. The cystic or neoplastic nature of KOs has been widely discussed in the literature. In 2017, WHO reversed the tumor classification announced in 2005 for cyst because many articles showed that the mutation in the PTCH gene could be found in non-neoplastic lesions and the resolution of the cyst after marsupialization was not compatible with a neoplastic process. In the same edition, OOC was placed as a separate entity from OK, because they are not associated with any syndrome, do not present a high rate of recurrence or aggressive clinical behavior. [28,12,31]

In a previous study, Okamoto and collegues. also evaluated the expression of podoplanin in OKs, OOCs and dentigerous cysts and observed negative expression in the basal layer of 73% of OOCs and weak expression in 27% when inflammatory cells were present. On the other hand, in 82% of the OKs the expression of podoplanin was strong, suggesting a possible contribution of podoplanin in the neoplastic nature of these lesions. In the present study, we analyzed the whole extent of the epithelial linning of all lesions and observed weak expression of OOCs in areas without inflammation[17].

Previous studies have shown that podoplanin is mainly expressed in peripheral cells (invasion front) of odontogenic tumors and in regions showing high mitotic activity, indicating that podoplanin expression is correlated with tumor invasion and cell proliferation [11,12,16, 32]. Moreover, during tooth development, high podoplanin expression was observed in enamel epithelial cells and cervical loop cells, which show high mitotic index, and weak podoplanin expression was observed in stratum intermedium and stellate reticulum cells, which show low proliferation index[33].

In our study, podoplanin expression was predominantly weak in the suprabasal layer of OOC, OK and NBCCS lesions. However, in 33.3% of NBCCS and in 14.3% of OKs the podoplanin expression was strong. These results indicate distinct proliferation potential in the suprabasal layers of some lesions. The higher proliferation potential in NBCCS lesions than those in the suprabasal layer of OK lesions, this could justify the more aggressive behavior that in isolated OKs cases.

Combined analysis of the basal and supra basal layers showed significantly stronger podoplanin expression in OK and NBCCS lesions than in OOC lesions, suggesting its participation in the processes of development and proliferation of odontogenic tumors, as indicated in other studies[13]. Singhal et al in 2017, observed the positive expression of podoplanin in odontogenic epithelium of ameloblastomas

and OKs when compared to dentigerous cysts and dental follicles, suggesting that a podoplanin would influence a proliferative activity, potentializing the intrinsic growth and locally invasive, which would justify a tumor nomenclature.[12]

It is interesting to note that in parallel, recent studies have shown the involvement of podoplanin in the activation of fibroblasts associated with malignant or potentially malignant oral lesions and also a significant expression of podoplanin in these lesions when compared to normal mucosa [14,15,27].

In 2016, Sindhu *et al* suggested that podoplanin-mediated cell migration and invasion are associated with cytoskeletal reorganization[34]. Moreover, podoplanin-induced migration of odontogenic cells is suggested to depend on the interaction of podoplanin with ezrin. Ezrin, which is belongs to the ERM protein complex, is essential for cellular physiological processes such as maintenance of cell shape, regulation of actin cytoskeleton, adhesion, and motility. Binding of ezrin to podoplanin may trigger a signaling pathway that induces projection formation, thus contributing to increased cell motility[18,35]. Oliveira et al, reported strong podoplanin and ezrin expression in the basal layer of OK lesions, suggesting that both ezrin and podoplanin contribute to the expansive growth and invasive potential of OKs[16].

Several studies have reported increased podoplanin expression in benign tumors that show invasion and recurrence characteristics and have suggested the role of podoplanin in tumor progression [9,11-13,16,17,27,34]. Zustin et al have previously demonstrated that podoplanin appears to be involved in the orthologic and pathologic processes of the formation of elongated cell extensions and odontoblastic fibers, in the epithelial-mesenchymal transition and local invasion during tooth germ development as well as in both reactive and neoplastic odontogenic cystic lesions[36]. Results of the present study indicate the clinical behavior of OK, NBCCS, and OOC lesions, with OK and NBCCS lesions being more aggressive and showing higher recurrence rate than OOC lesions. The pattern and location of podoplanin expression suggest that podoplanin participates in cell proliferation and migration by modifying the cytoskeleton. These lesions are rare, the number of samples is still limited and further studies with odontogenic tumors are needed to increase the precision of the conclusions drawn in comparative studies, especially with syndromic OKs.

The clarification of the exact role of this protein in cellular behavior and its interaction with other proteins may help in the development of new strategies for the

treatment of patients with NBCCS who may develop new lesions in different locations and in the future establish more efficient therapeutic alternatives.

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4 CONCLUSÃO

Os resultados do presente estudo correspondem de forma coerente ao que vem sendo apresentado na literatura científica, sendo as lesões CO e SCNCB, de comportamento mais agressivo, as de maior expressão de podoplanina. Além disso, o padrão e a localização da expressão da podoplanina poderiam sugerir a sua influência em atividades de proliferação e migração celular. A limitação do presente estudo se deve ao número reduzido da amostra, principalmente de COOs. Por outro lado, a inclusão no estudo de lesões ceratocísticas associadas a Síndrome do Carcinoma Nevóide de Células Basais, para a comparação da expressão dessa proteína, é um diferencial e reforça a associação da podoplanina com a proliferação e a invasão celular e a alta taxa de recidiva. Mais estudos são necessários para elucidar o mecanismo de ação dessa proteína no comportamento celular e sua interação com outras proteínas. O esclarecimento do papel exato dessa proteína pode auxiliar no desenvolvimento de novas estratégias de tratamento para os pacientes com SCNCB.

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