Keratocyst of the buccal mucosa: case report and immunohistochemical comparative study with sporadic intraosseous keratocystic odontogenic tumor

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Objectives. Describe a new case of keratocyst of the buccal mucosa and compare its immunohistochemical features with 13 sporadic intraosseous keratocystic odontogenic tumors (KOT).
Case Report and Study Design. A male complaining about an enlargement on the left buccal mucosa was referred to the Stomatology Clinic. Clinical examination revealed a solitary nodule posterior to the parotid papilla. An excisional biopsy was performed following clinical diagnosis of epidermoid cyst. Microscopically, the lesion was characterized by a lining of five cell layers, with columnar basal cells and a corrugated parakeratinized surface. Immunohistochemical reactions for PTCH-1, Smo, Shh, mTOR, bcl-2, Ck17, and Ck19 were performed. PTCH-1 was not expressed in the keratocyst of the buccal mucosa, but was observed in suprabasal layers of eight (61.5%) cases of sporadic intraosseous KOT. Shh, mTOR, bcl-2, Ck17, and Ck19 expression was observed in all the cases investigated.
Conclusions. The morphology and immunoprofile of this lesion are similar to sporadic intraosseous KOT. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:e387-e392)

Keratocystic odontogenic tumor (KOT) is a benign, uni or multicystic, intraosseous tumor that presents aggressive, infiltrative behavior. Microscopically, this tumor is characterized by an epithelial lining of 5-8 cell layers, cuboidal or columnar basal cells and a corrugated surface showing parakeratinization. The nuclei of the columnar basal cells exhibit reverse polarization and are often intensely basophilic.[1] Mitoses can be present in the basal and suprabasal layers.[1,2]

Solid-cystic,[3] peripheral[4] and intraosseous[1] variants of sporadic KOT have been described. Rare cases of parakeratinized cyst arising from the soft tissue, in the skin,[5] temporomandibular joint[6] and buccal mucosa,[7,8] with histopathological features identical to intraosseous KOT have been reported.[5-8] The origin and nature of this enigmatic lesion are controversial.[8] However, the histogenesis of intraosseous KOT is generally agreed to be odontogenic and evidence indicates 2 main sources of epithelium: dental lamina or its remnants and extensions of basal cells from the overlying oral epithelium.[1]

The pathogenesis of sporadic intraosseous KOT and basal cell nevus syndrome (BCNS) appear to be related to defects in the activation of the Hedgehog (Hh) signaling pathway caused by *PTCH-1* inactivation.[9,10] PTCH-1 protein is a receptor for the Sonic Hedgehog (Shh) protein, PATCHED-1 (PTCH-1) inhibits the signaling by repressing Smoothened (Smo) activity that leads to nuclear Gli activation.[9] This mechanism is a key regulator of embryonic development controlling cell proliferation and differentiation, particularly in the orofacial region.[9-11]

The aim of this study was to describe a new case of keratocyst of the buccal mucosa and compare the immunohistochemical features of this lesion with sporadic intraosseous KOT. We attempted to elucidate the origin and pathogenesis of the keratocyst in the buccal mucosa using a panel of antibodies against proteins of the Hh pathway including: PTCH-1, Smo, Shh mammalian target of rampamycin (mTOR), B-cell lymphoma-2 (bcl-2), Cytokeratin (Ck) 17 and Ck 19.

REPORT OF CASE AND METHODS

A 37-year-old Caucasian male was referred to the Stomatology Clinic at the State University of São Paulo (UNESP), São José dos Campos, Brazil, because of an enlargement of the left buccal mucosa that had been causing slight discomfort during mastication. His medical history revealed hypercholesterolemia. On clinical examination, a solitary submucosal nodule, tender on palpation, located in the left buccal mucosa posterior to

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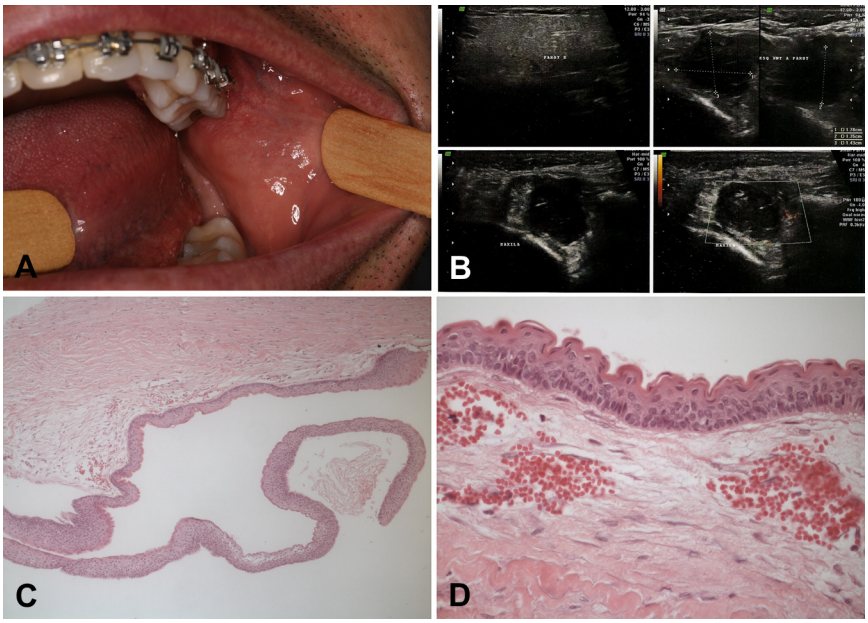

Fig. 1. **A,** Clinical aspect of the keratocyst of the buccal mucosa. **B,** Ultrasound and Doppler exam imaging. **C,** Photomicrograph showing the cystic lesion (HE stain, original magnification ×100). **D,** The keratocyst of the buccal mucosa lined by epithelial cell layers composed of columnar basal cells and presenting a corrugated parakeratinized surface (HE stain, original magnification ×400).

the parotid papilla (Figure 1*A*) was observed. Orthodontic appliances were fixed to correct a malocclusion.

Imaging exams were obtained. The ultrasound exam showed a well-delineated, hypoechoic, unilocular lesion measuring 2 × 1.5 cm (Figure 1*B*). Color Doppler imaging revealed scarce vascularization. Preoperative fine needle aspiration was performed. The clinical diagnosis of epidermoid cyst was suggested and an excisional biopsy was performed. Microscopically, the surgical specimen was characterized as a cyst lined with 5 cell layers composed of columnar basal cells and a corrugated parakeratinized surface (Figure 1*C* and *D*). The final diagnosis was a keratocyst of the buccal mucosa, in accordance with Ide et al. (2010).[8] No clinical evidence of recurrence was observed at 12 months postsurgery. Patient follow-up is ongoing.

IMMUNOSTAINING

The study sample consisted of 1 keratocyst of the buccal mucosa and 13 cases of sporadic intraosseous KOT. Slides stained with hematoxylin and eosin (HE) were revised by the authors (E.K., Y.R.C.), independently.

Three μm sections were deparaffinized and rehydrated in serial ethanol solutions. Antigen retrieval was performed in a pressure cooker in 10 mM citric acid solution (pH 6.0). Tissue sections were quenched of endogenous peroxidase by 3% hydrogen peroxidase (Merck, Rio de Janeiro, RJ, Brazil), followed by a washing step with 10 mM phosphate-buffered saline (pH 7.4) for 5 min. Incubations with primary antibodies: PTCH-1, dilution 1:400 (clone 5c7; Novus Biologicals, Littleton, CO, USA); Smo, dilution 1:200 (polyclonal; US Biological, Swampscott, MA, USA); Shh dilution 1:200 (clone EP1190Y; Abcam, Cambridge, MA, USA); mTOR, dilution 1:200 (polyclonal; Millipore, Billerica, MA, USA); bcl-2, dilution 1:600 (clone 124; Dako, Glostrup, Denmark); Ck17, dilution 1:50 (E3; Dako, Glostrup, Denmark) and Ck19, dilution 1:800 (Abcam, Cambridge, MA, USA) were performed. Reactions were developed with Post Primary Block, NovoLink Max Polymer (Novocastra Laboratories, Newcastle Upon Tyne, UK) for 30 min at 37°C and were visualized using DAB chromogen (Diaminobenzidine; Dako, Carpinteria, CA, USA). The sections were counterstained with Harris' hematoxylin. Positive and negative controls were included in all reactions. The positivity of the cells was independently determined in a blinded analysis by the authors (E.K., R.M.R. and Y.R.C.). The epithelium lining of KOT was histologically divided into basal and suprabasal layers. The clinicopathological features of 7 cases of primary keratocyst of soft tissue are presented in Table I.

RESULTS

Immunohistochemical analyses

PTCH-1 was not expressed in the keratocyst of the buccal mucosa, but was observed in suprabasal layers of eight (61.5%) cases of sporadic intraosseous KOT. When PTCH-1 was absent, Smo expression was observed. No differences in Shh expression were noted between the cases of KOT, which was present in both