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Case report

Immune cell infiltration in gingival epithelioid angiomatous nodule: Case report and immunohistochemical analysis☆,☆☆

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ABSTRACT

Epithelioid angiomatous nodule (EAN) is a rare benign vascular proliferation, regarded as part of the morphologic spectrum of benign and malignant epithelioid vascular lesions. EAN is a rare lesion affecting the oral mucosa and, to date, only three cases have been reported in the English-language literature. We report the second EAN case affecting the gingival mucosa of a 69-year-old female patient. Oral examination revealed an asymptomatic, well-defined nodule exhibiting a smooth and erythematous surface, measuring 0.8 cm in greater diameter. The lesion was fully excised and histopathological study showed a mucosal epithelioid proliferation with solid and organoid growth patterns, and vascular lumens scattered focally throughout the lesion. The large epithelioid cells showed intracytoplasmic vacuoles and vesicular nuclei with prominent nucleoli, surrounding by scarce extravasated erythrocytes. Immunohistochemistry showed positivity for vimentin, α-SMA, CD34, focally for D2-40, and Ki-67 was 15%. Noteworthy, numerous immune cells (HLA-DR+/CD68+/CD163+/FXIIIa+) scattered throughout the lesion, were detected. To the best of our knowledge, this is the first report, which highlights the immune cell population, with M2-like phenotype, as an important component of EAN, suggesting the participation on their etiopathogenic mechanisms.
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1. Introduction

Epithelioid angiomatous nodule (EAN), is a rare benign vascular proliferation, firstly described in the skin by Brenn and Fletcher, and clinically characterized by a nodule or papule well circumscribed [1,2]. The color can vary from erythematous to violaceous, and it can be painful [3]. EAN primarily affects young adults, aged between 15 and 45 years, with no gender preference; however, one study has noted male predominance [3–5]. In the first descriptions, trunk and extremities were reported as the sites most commonly affected [5]. Currently, the head and neck region is considered the most predominant anatomic site [5]. The etiology of EAN is unknown, but its rapid and ultimately self-limited growth suggests that it is probably a reactive or benign process [6]. To date, no EAN case showing recurrence, progression or metastasis has been reported [3].

EAN is regarded as part of the morphologic spectrum of benign and malignant epithelioid vascular proliferations that includes epithelioid hemangioma (EH), epithelioid hemangioendothelioma (EHE) and epithelioid angiossarcoma (EA) [3,6]. Microscopically, EAN is characterized by a solid and clearly circumscribed proliferation of epithelioid cells that may present vacuoles in the cytoplasm, without cellular atypia or pleomorphism, but some degree of typical mitosis can be visualized [7]. In the oral mucosa, EAN is a rare lesion, with only three previously published cases, in which one

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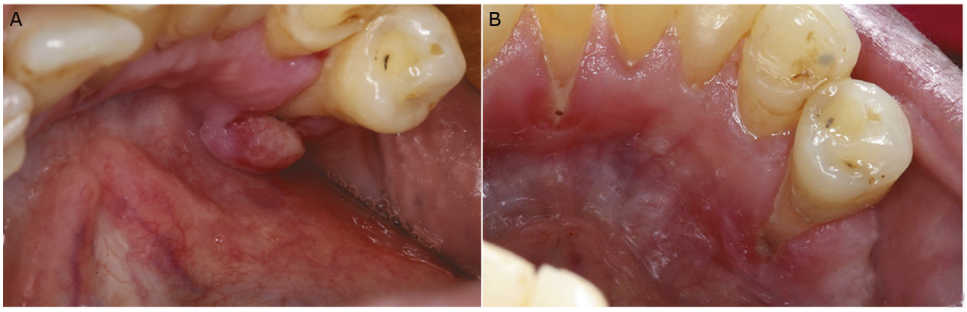

Fig. 1. Clinical aspects of EAN. Clinical view showing a well circumscribed nodule on the mandibular gingiva at level of the right first premolar, diagnosed as EAN (A). Clinical view 1 year after excisional biopsy (B).

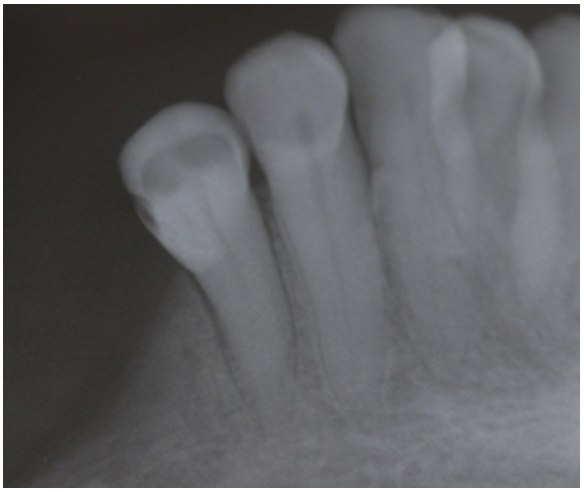

Fig. 2. Radiographic aspects of EAN. A radiographic view showing preservation of the periodontal tissues, confirming restriction to soft tissue.

of them affected the gingival mucosa [5,8]. Moreover, there are two EAN cases affecting each one the skin of upper and lower lip [5]. The distinction of EAN from other epithelioid vascular lesions can be made on the basis of the clinical and morphological features. However, in some cases, immunohistochemistry is helpful for establishing the correct diagnosis, which highlights the endothelial cells and smooth muscle cells, as the major components of the lesion. Interestingly, infiltrating non-neoplastic immune cells in EAN has not been reported so far. Thus, the aim of this paper is to report the second case of EAN located in the gingival mucosa and to review the English-language literature. To the best of our knowledge, this is the first report which highlights the intralesional immune cell population, with a M2-like phenotype, as an important component of EAN, suggesting participation on their etiopathogenic mechanisms.

2. Case report

A 69-year-old, otherwise healthy, Brazilian woman was referred presenting a lesion of 2-month evolution on the lingual marginal gingiva of the mandibular right first pre-molar. Intraorally, was observed an asymptomatic well-defined nodule, with a smooth and erythematous surface, measuring 8 millimeters in greater diameter, which clinically was diagnosed as pyogenic granuloma (PG), peripheral giant cell lesion (PGCL), peripheral ossifying fibroma (POF) or peripheral odontogenic tumor (Fig. 1A). Periapical radiograph shows no bone involvement (Fig. 2). An excisional biopsy was performed, and histopathological analysis showed a mucosal epithelioid proliferation with solid and organoid growth patterns, which exhibited scattered throughout the lesion, small vascular lumens of varying caliber. In fact, intralesional vascular channels, lined by a monolayer of epithelioid endothelium, were frequently observed. In high-power view, the large epithelioid cells showed intracytoplasmic vacuoles and vesicular nuclei with prominent nucleoli, admixed with scarce granulocytes and extravasated erythrocytes, and supported by fine connective tissue network (Fig. 3). Immunohistochemistry showed positivity for vimentin, α-SMA, CD34, focally for D2-40, and Ki-67 labeling index was 15%. CD138 evidenced few plasma cells and discreetly the cellular stroma, while that desmin highlighted focally the vascular wall. AE1/AE3 pan-cytokeratin, EMA, p53, p63 and Bcl-2 were negative (Fig. 4). Noteworthy, numerous immune cells (HLA-DR+/CD68+/CD163+/FXIIIa+), scattered throughout the lesion, in perivascular pattern, were visualized. Moreover, CD1a and CD207 markers were practically negative, whereas scarce S100 positivity was detected, indicating absence of Langerhans cells (Fig. 5).

At present, the patient is under clinical follow-up, and after 1 year, no recurrence or alteration has been observed.

3. Discussion

Epithelioid angiomatous nodule is a rare benign vascular proliferation, firstly described by Brenn and Fletcher in 2004, in a series of 15 cases [1]. EAN is extremely rare on the oral mucosa. Since this lesion was initially described, only three oral EAN cases have been reported [5,8] and the current case appears to be the second affecting the gingiva. Table 1 summarizes the clinical features presented in case reports described to date in the literature Moreover, there are two EAN cases affecting each one the skin of the upper and lower lip [5]. In the oral cavity, two lesions were located in the tongue and one in the maxillary gingiva. The size varied from 0.3 to 0.8 centimeters and the time the evolution from two weeks to one month. There were two women and one man, being the mean age 27.6 years (range, 13–49 years). Clinically, EAN is characterized by a nodule or papule well circumscribed. The color can vary from erythematous to violaceous and it can be painful [3]. In the present case, the lesion was an asymptomatic well-defined nodule, located on the mandibular gingiva, measuring 0.8 centimeters in greater diameter, and with 2-month evolution, which clinically was diagnosed as PG, PGCL, POF or peripheral odontogenic tumor. The radiographic analysis did not show alterations, showing that the lesion was restricted to gingival mucosa. Clinically, PGs are reactive lesions and occur often in traumatized areas as face and oral cavity. Histologically are constituted by a lobular proliferation of capillaries consisting of endothelial cells, surrounded by pericytes in a loose stroma, which contains numerous inflammatory cells. In the periphery, ulceration is frequent [6,9]. For both, EAN and PG, the treatment consists in simple local excision [5]. PGCL is a reactive lesion, originates from the periosteum or periodontal ligament [10,11]. Histologically is constituted by numerous multinucleated giant cells admixed with mononuclear cells, hemorrhagic focuses

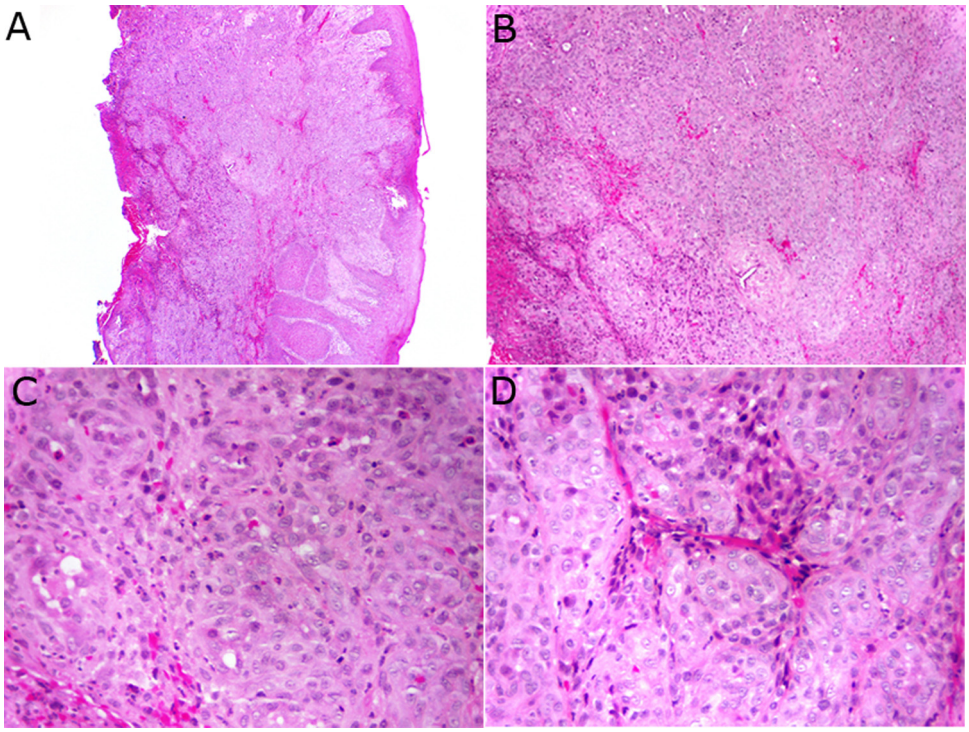

Fig. 3. Histopathological features of the EAN (H&E stain). A mucosal epithelioid proliferation with solid and organoid growth patterns throughout the lesion (A, x4; B, x10). In high-power view, notice the intralesional vascular channels and large epithelioid cells showing intracytoplasmic vacuoles, admixed with scarce granulocytes and extravasated erythrocytes (x40).

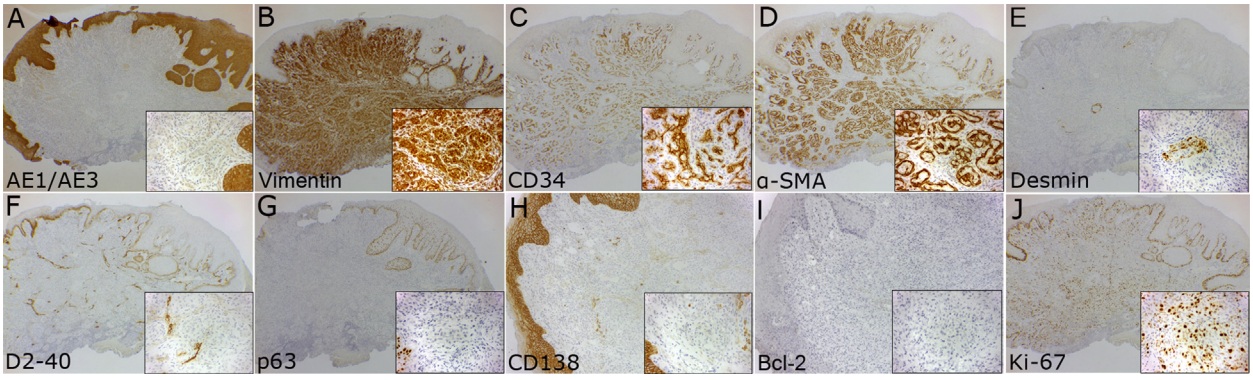

Fig. 4. Immunohistochemical analysis of EAN. EAN was strongly positive for vimentin (B), CD34 (C) and α-SMA (D). D2-40 positivity was focal (F), CD138 evidenced few plasma cells (H), while desmin highlighted one vascular wall (E). AE1/AE3 (A), p63 (G) and Bcl-2 (I) were negative. The Ki-67 labeling index (J) was 15% (A-G, J, x4; H, I, x10; all inset, x40).

Table 1
Summary of cases of EAN in the oral cavity and lip.

Reference	Age	Sex	Site	Size (cm)	Duration	Clinical Diagnosis	Treatment	Follow-up (M)
Ide et al. [8]	49	F	Maxillary gingiva	0.8	2 W	PG	Exc	60
Sun et al. [5]	21	M	Tongue	0.3	1 M	NA	Exc	NA
	45	F	Lower lip (skin)	0.5	1 M	PG	Exc	72
	13	F	Tongue	0.3	1 M	PG	Exc	36
	34	M	Upper lip (skin)	1.2	6 M	Vascular lesion	Exc	12
Present case	69	F	Mandibular gingiva	0.8	2 M	PG	Exc	12

Exc: excision; F: female; M: male; M: months; NA: not available; PG: pyogenic granuloma; W: weeks.

and hemosiderin deposits [10,12]. POF is also a reactive lesion exclusively affecting the gingiva. Histologically their particular feature is the presence of mineralized tissue supported by fibrous stroma [10,13]. All these lesions are best treated by complete surgical excision, as well as elimination of the predisposing factors, such as poor oral hygiene, trauma, badly finished restorations, calculus and impacted food [10,11]. Peripheral odontogenic tumors are rare lesions that correspond to 4.3% of all odontogenic tumors [14].

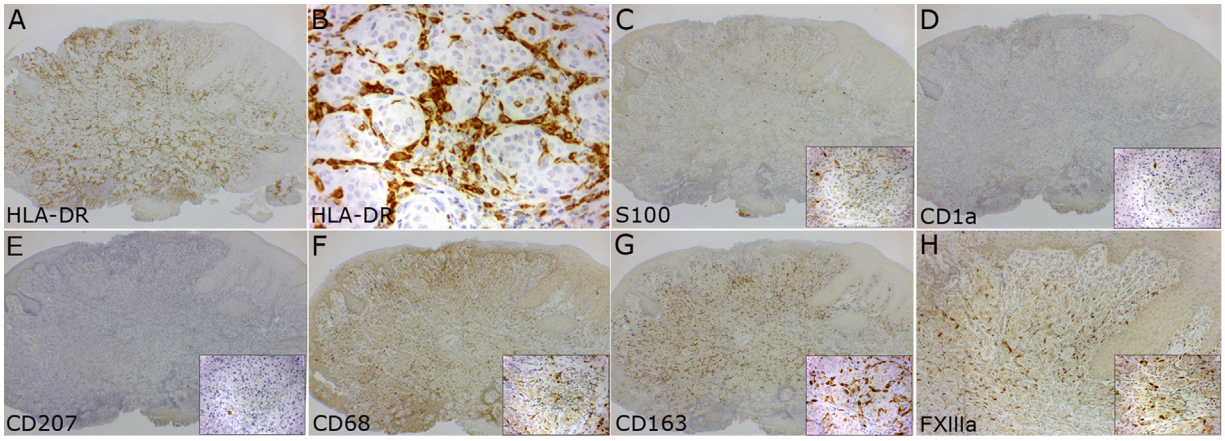

Fig. 5. Immunohistochemical analysis of EAN. EAN showing extensive intralesional dendritic-like immune cell infiltration. Assessing consecutive labeled slides, these intralesional immune cells revealed positivity for HLA-DR (A, B), CD68 (F), CD163 (G) and FXIIIa (H). Moreover, scarce cells were S100 positive (C), while CD1a (D) and CD207 (E) were negative (All but B, x4; B and all inset, x40).

The most common tumors are peripheral odontogenic fibroma and peripheral ameloblastomas [15]. Peripheral odontogenic fibroma, which represents the soft tissue counterpart of central odontogenic fibroma, is a benign odontogenic neoplasm, histologically presentating mature collagenous fibrous tissue in addition to odontogenic epithelium [10]. Likewise, peripheral counterpart of central lesion, the peripheral ameloblastomas are lesions of indolent behavior and microscopically exhibit the same histopathological subtypes from central lesions [16].

Microscopically, the differentiation of EAN from mimickers is complex and the differential diagnosis should include epithelioid hemangioma (EH), epithelioid hemangioendothelioma (EHE) and epithelioid angiosarcoma (EA) [6]. EH typically appears as a single or multiple nodule on the head and neck region of adult males. Histologically, EH is similar to EAN, but vasoformative nodules with vessels lined by epithelioid endothelial cells and a dense inflammatory infiltrate with lymphoid follicles and abundant eosinophils are more prominent in EH [3,9]. EHE and EA show typical clinical characteristics of a malignant tumor. Histopathologically, EHE is constituted by nonvasoformative nodules with epithelioid cells arranged as cords and strands integrated in a fibromyxoid stroma. There are also mitotic figures and cellular pleomorphism. EA presents ill-defined proliferation of epithelioid endothelial cells with ample cytoplasm and vesicular nuclei with prominent nucleoli arranged as solid sheets. It presents high levels of atypia, pleomorphism and aberrant mitoses, not seen in EAN [3].

In the current case, interestingly, we observed peculiar immunohistochemical features. While vimentin was strongly positive, CD34 and α-SMA highlighted epithelioid endothelial cells and pericytes, respectively (Fig. 4C, D). Notably, this pattern is unusual in EHE and EA. In fact, these two latter show a diffuse immunopositivity for endothelial markers mainly, and different from our case, the positivity for α-SMA is weak, highlighted probably stromal myofibroblasts. On the other hand, the D2-40 immunomarker in our case showed a focal, scattered positivity in the lymphatic vessels throughout the lesion, as well as uniform positivity in the basal layer of the surface epithelium (Fig. 4F). Differently from our case, a cutaneous EAN showed D2-40 positive vessels that were more peripherally placed in the nodule, failing to express D2-40 in the central areas of the lesion [17]. The author suggested that this pattern of immunostaining with D2-40 contrasts with the one described in angiossarcomas, which is diffuse. Nevertheless, our findings do not support this proposal.

Moreover, among the lesional cells, the current EAN case showed numerous dendritic-like cells highlighted by HLA-DR, CD68, CD163 and FXIIIA, isolated cells were S100 positive, while that CD1a and CD207 were practically negative (Fig. 5). To the best of our knowledge, we report for the first time, that EAN may present infiltration by numerous dendritic-like immune cells, which exhibited the HLA-DR+/CD68+/CD163+/FXIIIA+ immunophenotype. HLA-DR molecules are typically found on macrophages and dendritic cells, being that these molecules are often increased in response to immune stimulation [18]. Both CD68 and CD163 immunoexpression on these dendritic-like immune cells is consistent with that described for tumor-promoting M2-like macrophages, which provide growth and pro-angiogenic factors and show potent immunosuppressive functions [19]. FXIIIA+ cells represent a heterogeneous population of bone marrow-derived dendritic-like cells, distinct from Langerhans cells, which share some features common to macrophages [20]. Thus, an intermediate immunophenotype between macrophages and dendritic cells for the infiltrating immune cells in our EAN case should be considered.

Analysis of intralesional immune cell infiltration in EAN cases is recommended to validate our findings. Moreover, if infiltration by immune cell populations occurs in the spectrum EH, EHE and EA are unknown. Studies regarding this topic could provide valuable information about the interaction between tumor or lesional cells with immune cells and surrounding stroma, which will certainly also supply data for a detailed cellular characterization in EAN cases and the spectrum EH, EHE and EA.

In summary, we report the second case of gingival EAN which exhibited an infiltrating immune cell population, with M2-like phenotype, as an important component of EAN, suggesting participation on their etiopathogenic mechanisms.

Conflicts of interest

The authors report no conflicts of interest related to this letter.

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