Original Research
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The role of Osterix protein in the pathogenesis of peripheral ossifying fibroma

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Abstract: Peripheral ossifying fibroma (POF) is a reactive lesion of oral tissues, associated with local factors such as trauma or presence of dental biofilm. POF treatment consists of curettage of the lesion combined with root scaling of adjacent teeth and/or removal of other sources of irritants. This study aimed to analyze the clinical and pathological features of POF and to investigate the immunoexpression of Osterix and STRO-1 proteins. Data such as age, gender, and size were obtained from 30 cases of POF. Microscopic features were assessed by conventional light microscopy using hematoxylin-eosin staining and immunohistochemical markers, and by polarized light microscopy using Picrosirius red staining. The age range was 11-70 years and 70% of the patients were female. Moreover, the size of POF varied from 0.2 to 5.0 cm; in 43.33% of the cases, the mineralized content consisted exclusively of bony trabeculae. The immunohistochemical analysis showed nuclear staining for Osterix in 63% and for STRO-1 in 20% of the cases. Mature collagen fibers were observed in mineralized tissue in 76.67% of the cases. The clinical and microscopic features observed were in agreement with those described in the literature. Osterix was overexpressed, while STRO-1 was poorly expressed. Osterix was expressed particularly in cells entrapped in and around mineralized tissue, indicating the presence of a stimulus that triggers the differentiation of these cells into osteoblasts or cementoblasts, *i.e.*, cells that produce mineralized tissue. Based on our results, Osterix may play a role in the pathogenesis of POF.

Keywords: Fibroma, Ossifying; Immunohistochemistry; Collagen; Osteogenesis.

Introduction

Peripheral ossifying fibroma (POF) is a lesion of oral tissues that accounts for about 20% of all non-neoplastic oral proliferative processes. The condition is more prevalent among women and affects mainly the gingiva, especially in the anterior region of the jaw.[1] However, cases of POF at edentulous sites have been reported.[2] POF appears as a solitary nodule of slow and progressive growth,[1] although there have been reports of multifocal POF.[3] Radiopaque areas can be identified on radiographs depending on lesion size and on the presence of mineralized foci.[4] Microscopically, POF is characterized by

fibrocellular connective tissue, composed of ovoid or spindle-shaped fibroblasts with a vesicular nucleus,[5] covered by stratified squamous epithelium that can be ulcerated. Infiltrates of lymphocytes, plasma cells, macrophages, and giant cells,[4] as well as collagen and bony trabeculae and/or cementum-like material are also observed.[5] POF arises from periodontal ligament cells, and local factors such as trauma, dental biofilm, calculus, and microorganisms have been associated with this condition.[6]

Osterix (OSX) is essential for the differentiation of preosteoblasts into mature osteoblasts, and one study showed that it might be stimulated by estrogen.[7] OSX is also expressed in osteoblasts[8] and cementoblasts.[9] The STRO-1 marker allows the identification of stem cells with a high capacity for proliferation and differentiation into osteoblasts and cementoblasts (*e.g.*, those found in the periodontal ligament).[9,10]

The recurrence rate of POF is approximately 20%.[11] To minimize treatment failure, the lesion must be removed completely; root scaling of adjacent teeth and/or removal of other source of irritants is also indicated.[4,11,12] When the maxillary anterior area is affected, complete excision can result in significant esthetic problems.[4] The aim of this study was to analyze the clinical and microscopic features and to investigate the immunoexpression of OSX and STRO-1 in POF.

Methodology

The pathological and clinical records of 30 patients with primary POF diagnosed at the Oral Pathology Laboratory of our institution between 2000 and 2010 were reviewed. Data such as age, gender, and location and size of the lesions were collected. Slides of the respective cases were stained with hematoxylin-eosin (H&E) for light microscopy analysis, and all slides were reviewed for POF considering the presence of connective tissue and calcifications. Mineralized contents were analyzed according to the architectural pattern, presence of osteoblastic rimming around mineralized tissue, and presence of osteoclasts. The non-mineralized part of the lesion was evaluated for vascularization, morphology, and fibroblast arrangement. The slides were subjected to immunohistochemistry for analysis of OSX and STRO-1 expression, and stained with Picrosirius red for polarized light microscopy.

Polarized light microscopy

The slides were stained with Picrosirius red (Abcam, Cambridge, USA) for analysis of fibrillar and mineralized matrix maturation under a polarized light microscope (Axioplan, Zeiss, Göttingen, Germany). The maturation pattern was categorized as red/orange for mature fibers and as green for immature fibers.[13]

Immunohistochemistry

Three-micrometer sections were deparaffinized in xylene and hydrated in decreasing grades of alcohol solutions. The slides were subjected to antigen retrieval methods according to the antibody of interest, which differed in terms of the procedures and buffer solutions used. Endogenous peroxidase was blocked by means of a 6% hydrogen peroxide solution (Merck, Darmstadt, Germany), followed by immersion in TRIS buffer (pH 7.4) for 15 minutes at room temperature. After that, the slides were incubated with the primary antibody against OSX and STRO-1 (Abcam, Cambridge, USA) and diluted (1:100) in ready-to-use diluent (DakoCytomation, Glostrup, Denmark), at room temperature, for 16 hours. A commercial streptavidin-biotin-peroxidase system was used to detect the reaction, in accordance with the manufacturer's specifications (LSAB, DakoCytomation, Glostrup, Denmark). The reaction was developed using diaminobenzidine (DakoCytomation, Glostrup Denmark) as chromogenic substrate. Finally, the sections were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany).

The results were analyzed independently by three observers using a 40x objective and were classified as follows: negative, 0–10% of staining; and positive, > 11% of staining in five different randomly chosen fields.

Statistical analysis

Associations between immunoexpression and the clinicopathological features in 2 x 2 contingency tables were evaluated using Fisher's exact test and a level of significance of 5%. The Minitab software

(Minitab, Inc., State College, PA) was used for the statistical analysis.

Results

Analysis of the medical records of the selected cases showed there were 21 (70%) women and 8 (30%) men. The age range was 11–70 years (mean: 33 years, median: 30 years, and standard deviation: ± 17.03). The lesions were predominantly located in the gingiva in 27 (90%) cases, including the free and attached gingiva or incisive papilla (Figure 1A), and in the alveolar ridge in 3 (10%) cases (Figure 1B). The lesions appeared as sessile and pedunculated nodules ranging in size from 0.2 to 5 cm.

Microscopically, epithelial hyperplasia was found in 57% of cases, accompanied by a mixed inflammatory infiltrate in the underlying connective tissue. The nodule was ulcerated in 33% of cases, with a predominance of polymorphonuclear inflammatory cells. In 43.33% of cases, the mineralized tissue consisted exclusively of bony trabeculae (Figure 2A); 14.33% exhibited only cementum-like tissue, and 43.33% of the lesions had both types of mineralized tissue. The bone was surrounded by osteoblastic rimming, and osteoclasts were found in 30% of cases. Mineralized tissue was present around the blood vessels in 3% of the cases analyzed (Figure 2B). The non-mineralized part of the lesion consisted of oval and spindle-shaped cells containing vesicular nuclei and evident nucleoli.

Polarized light microscopy

Polarized light microscopy was performed to evaluate the presence of mature and immature collagen fibers in mineralized tissue (Figure 3A) and non-mineralized tissue (Figure 3B) of the lesions (Table).

Immunohistochemistry

Nuclear staining of OSX was observed in 63% of the cases, and it was detected in spindle-shaped cells around the mineralized tissue, in entrapped osteocytes (Figure 4A), and in foci of mineralized tissue formation (Figure 4B). STRO-1 was expressed in

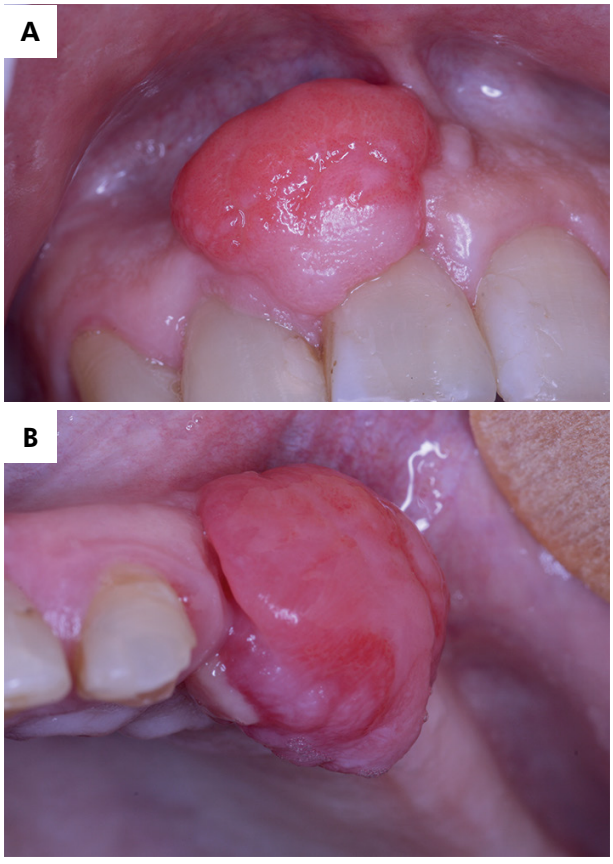

Figure 1. Clinical photograph of cases that occurred in the gingiva (1A) and alveolar ridge (1B).

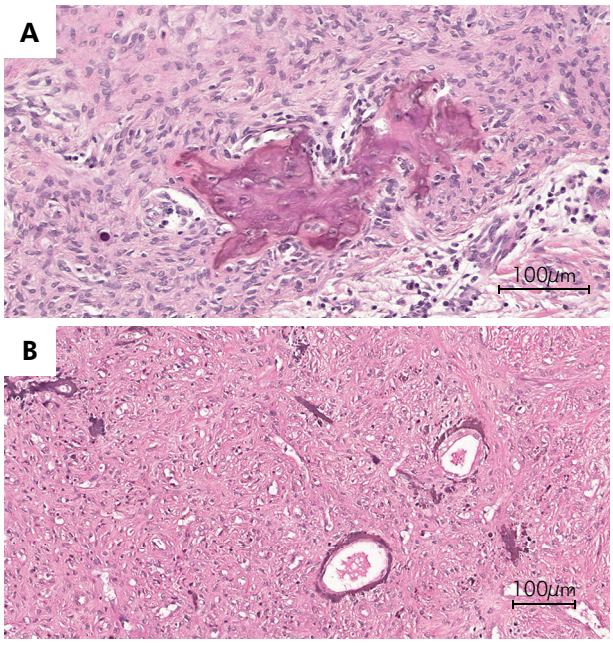

Figure 2. Photomicrographs: bony trabeculae in cell-rich tissue, HE(2A); presence of mineralized tissue around blood vessels, HE (2B).

20% of cases and was found near mineralized tissue, in entrapped osteocytes, and in foci of mineralized tissue formation (Figure 4C). No STRO-1 staining was observed in 70% of the cases (Figure 4D) and immunohistochemical staining was not analyzed in three cases because of the scarcity of material.

There was no significant correlation between immunoexpression and the clinicopathological features ($p \geq 0.05$).

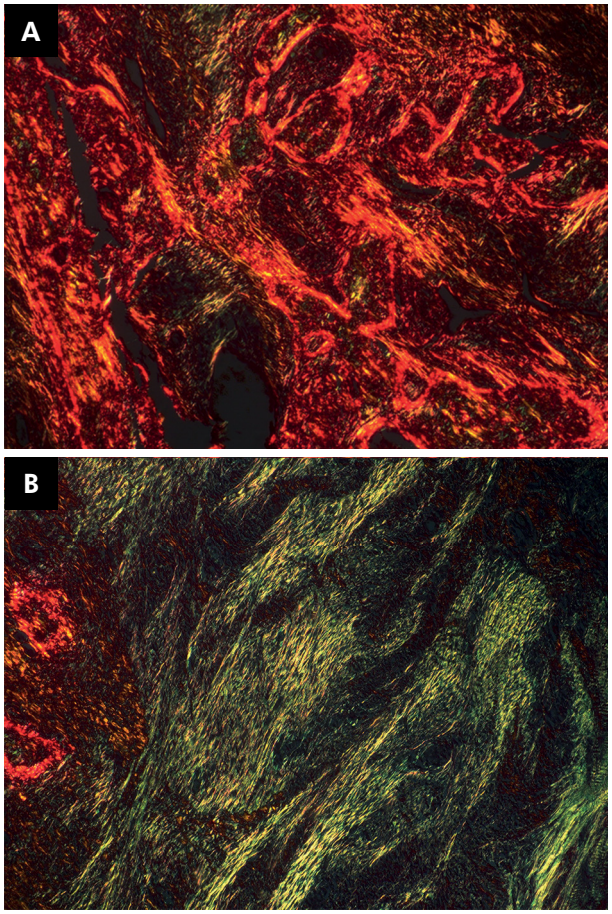

Figure 3. Polarized light photomicrographs: mature collagen fibers appear reddish and yellowish (3A) and immature collagen fibers appear greenish (3B), Picrosirius red staining, 100x.

Table. Maturation of fibers at each lesion site. n (%).

Type of fibers	Mineralized tissue	Non-mineralized tissue
Mature fibers	23 (76.67)	25 (83.33)
Immature fibers	2 (6.66)	5 (16.66)
Both	5 (16.66)	0 (0.00)

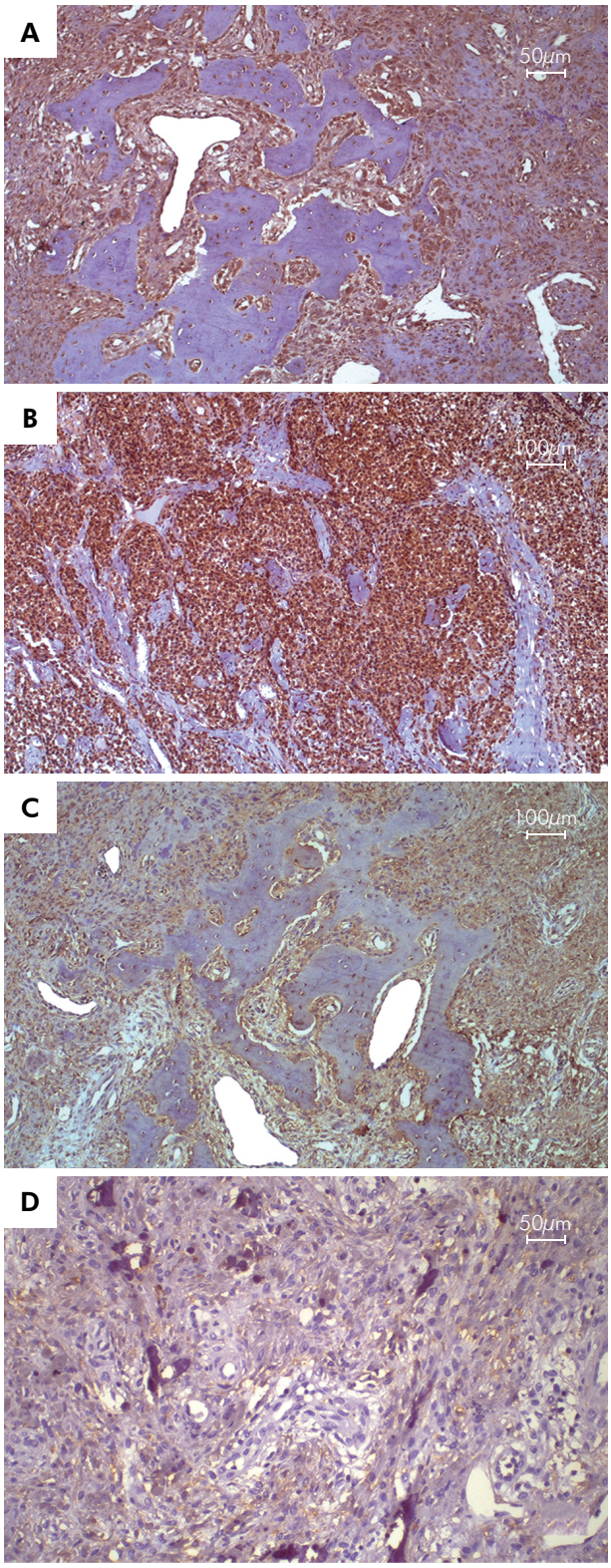

Figure 4. Photomicrographs: OSX indicating spindle-shaped cells around the mineralized tissue (4A) and OSX high immunopositivity in the cell-rich tissue (4B). STRO-1, indicating spindle-shaped cells around the mineralized tissue (4C) and negative stainning g of STRO-1 (4D). Mayer`s hematoxylin counterstain.

Discussion

Peripheral ossifying fibroma is a common lesion in the gingiva. The condition commonly affects women,[1] as was observed in this study. Patient age varied widely from 11 to 70 years in the present study when compared with the literature, which reports a higher prevalence in the second decade of life.[12] Sessile or pedunculated nodules of variable size were observed, similar to the reports of other authors.[4,12,14] POF exhibits clinical features akin to those of pyogenic granuloma, peripheral giant cell lesion,[14,15] and osteoblastoma.[16]

In the present study, epithelial proliferation accompanied by inflammation of the underlying tissue was a common finding, probably because inflammatory and connective tissue cells secrete cytokines and other chemical mediators that stimulate epithelial cell proliferation.[17] Bone trabeculae or rounded cement-like mineralized tissue was observed. Osteoblastic rimming was observed around the bone. Oval cells with a clear vesicular nucleus and spindle-shaped fibroblasts were found in the connective tissue adjacent to the mineralization. There were a small number of osteoclasts, as suggested in the literature,[18] although few studies have reported the presence of osteoclasts in POF. Polarized light microscopy revealed a considerable number of immature collagen fibers stained green in the non-mineralized tissue, possibly because of constant trauma, according to Kulkarni.[13] Picrosirius red staining and polarization microscopy help only to evaluate the thickness and, consequently, the degree of maturity[13] of the collagen fibers, which is a limitation of this study. To specify the type of collagen fiber present in the lesion, a complementary immunohistochemical test would be necessary.

STRO-1 is a cell surface antigen expressed by bone marrow precursor cells that is currently used to identify periodontal ligament stem cells.[10] STRO-1-positive cells in human bone marrow have the capacity to self-renew and to differentiate into smooth muscle cells, adipocytes, chondrocytes, and osteoblasts.[10] Positive STRO-1 staining may indicate secretion of bone matrix and of cementoid and dentinoid material.[19] STRO-1 has been associated with increased osteogenic capacity[20] and is a marker of stem cells.[21] Studies have demonstrated STRO-1 expression around vessels as well in the pulp[22] and also in pyogenic granuloma and POF.[23] In contrast with the literature, this study found less STRO-1 expression near mineralized tissue, suggesting that these few stained cells have not yet undergone differentiation into osteoblasts or cementoblasts.

OSX is a transcription factor with an important role in the differentiation of osteoblasts and cementoblasts[8,9] that occurs in odontogenesis and osteogenesis.[24] Tsiligkrou et al.[25] investigated the expression of proteins in POF such as Runx-2, bone morphogenetic protein 2 (BMP-2), and cementum attachment protein (CAP). They suggested that the cell populations present in the lesion belonged to mineralized tissue-forming cell lineages, i.e., the osteoblastic or cementoblastic types. Other research studies have demonstrated participation of OSX in the modulation that occurs in vascular calcifications, which affect mainly patients with chronic kidney disease.[26] This fact encourages further studies to investigate the relationship of this protein with blood vessels, since the present study observed calcifications near and around blood vessels. OSX was highly expressed in this study, particularly in cells entrapped in and around mineralized tissue, indicating the presence of a stimulus that triggers the differentiation of these cells into osteoblasts or cementoblasts, *i.e.*, cells that produce mineralized tissue. Based on the results of this study, OSX may participate in the pathogenesis of POF.

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