

Peripheral-Ossifying-Fibroma; Clinicopathological Study

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Abstract: Objectives: To investigate clinical and immunohistochemical characteristics of Peripheral ossifying fibroma (POF) cases, and to compare the results with normal & reactive lesions to clarify the nature of these lesions. **Methods:** Clinical, immunohistochemical and management of three cases of POF diagnosed and treated at the faculty of Dentistry, King Abdul Aziz University, Jeddah, Saudi Arabia, were presented & compared with specimens of normal oral mucosa and irritational fibroma. **Results:** Two males and one pregnant female patient presented to the college with recurrent clinically benign gingival lesions. The histological examination was consistent with POF. The immunohistochemical analysis of the cases was moderately to strongly positive to both vimentin and actin. However, specimens of normal oral mucosa and fibrous hyperplasia showed moderate positive reaction to vimentin in the fibroblast cells, with weak positive reaction to actin noted only in the cells of the blood vessel walls. **Conclusion:** The results represent further evidence of the possible myofibroblastic nature of the POF lesions, and are consistent with a possible origin from the periodontal ligaments. [Safia Al-Attas, Zeinab Darwish and Suzan Ibrahim. **Peripheral-Ossifying-Fibroma; Clinicopathological Study of 3 Cases.** *J Am Sci* 2012; 8(8):22-28]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 4

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Introduction

Peripheral ossifying fibroma (POF), is a gingival nodule composed of a cellular fibroblastic connective tissue stroma associated with the formation of randomly dispersed foci of mineralized tissues^(1; 2). In the literature POFs have been given several names, such as peripheral fibroma with calcification, calcifying fibroblastic granuloma, peripheral cementifying fibroma, and peripheral cemento-ossifying fibroma^(3; 4), which might indicate that there is much controversy concerning the classification of these lesions.

Clinically, POFs are sessile or pedunculated interdental lesions mainly affect women in the second decade of life and are usually located in the maxilla anterior to the molar area. Most lesions are less than 2 cm in size, although larger ones occasionally occur, causing extensive destruction of adjacent bone and significant functional or esthetic alterations⁽¹⁻⁵⁾. The diagnosis of the POFs based solely on clinical aspects can be difficult and histopathological examination is mandatory for definitive diagnosis^(5; 6). Histologically, POF shows a parakeratinized and hyperplastic epithelium and well-cellularized connective tissue containing mineralized components ranging from bone to cementum and, less frequently, dystrophic calcifications^(7; 8).

Treatment consists of conservative surgical excision and scaling of adjacent teeth. The recurrence rate of 8%–20% probably occurs due to incomplete initial removal or persistence of the local irritants⁽⁴⁾. The etiopathogenesis of POF has not been established yet; some authors still reported them as tumors⁽⁹⁾, while the majority considered them as reactive lesions frequently associated with irritant agents such as calculus, bacterial plaque, orthodontic appliances, ill-adapted crowns, and irregular restorations⁽²⁻⁵⁾. Overall, the mineralized product might be originating from periosteal cells or from the periodontal ligament^(6; 10). Hormonal influences may play a role, given the higher incidence of POF among females, increasing occurrence in the second decade and declining incidence after the third decade^(2; 3; 11). Finally genetic mutations have been suggested in the case of multicentric POF cases⁽¹²⁾.

Objective

To investigate clinical and immunohistochemical characteristics of POF cases, and to compare the results with normal and reactive lesions as well as with information available in the literature to clarify the nature of these lesions.

Case description

This is a retrospective study in which three cases of POFs that had been diagnosed, surgically excised, and submitted for histopathological examination by the faculty of Dentistry King Abdul-Aziz University, Jeddah Saudi Arabia, from the year 2000 up to 2012 were clinically and immunohistochemically studied. Data regarding patient's age, gender, history of recurrence as well as the lesion's anatomical location and clinical diagnosis were collected at Oral Medicine outpatient clinics. Exceptional soft tissue biopsy specimens were formalin fixed, embedded in paraffin, and subsequently cut into 5µm thick sections using a tissue microtome. Sections were mounted on routine glass slides for H&E evaluation, and on positively charged glass slides for immunohistochemical study. In addition, two specimens were included in the study, one from normal tissue and another was an archival biopsy of fibrous hyperplasia case (irritational fibroma). Normal tissue specimen was collected from consenting healthy patient who was scheduled for routine oral surgery clinics.

Informed consent was obtained from all patients before starting the study. Both the protocol and consent form were reviewed and approved by the ethical committee of the Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

The study was conducted in accordance with the ethical principles provided by the Declaration of Helsinki and according to the principles of good clinical practice.

Immunohistochemical technique

Slides of paraffin blocks were prepared for immunohistochemical analysis using a standard streptavidin- biotin staining method and two primary antibodies, antivimentin (monoclonal, clone M 7020, Dako, Denmark) and anti-specific muscle actin (α -SMA) (monoclonal, clone HHF35, Dako, Denmark). The sections were deparaffinized in 2 changes of xylene for 10 minutes each, and then were deparaffinized through graded alcohols and immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed in a steamer for 30 minutes in 10 mmol/L citrate buffer, pH 6.0. The primary antibodies, anti-vimentin and anti- actin were applied to the tissue sections for one hour. Biotinylated anti-immunoglobulin was used as a secondary antibody for 30 minutes. The slides then were incubated with the peroxidase conjugated streptavidin label for 30 minutes and with diaminobenzidine for 3 to 5 minutes. Counter staining was done using Mayer's hematoxylin. The negative control consisted of specimens in which the

primary antibody was replaced with normal mouse IgG at an appropriate concentration.

Staining evaluation

The results were semi-quantitatively evaluated as follows: negative (no staining), weak (focal staining of less than 30% of all spindle cells were positive), moderate (positive staining of more than 30% and less than 70% of all cells) and strong staining (more than 70% of all cells were positive). Only spindle cells were evaluated.

Results

Over the last eleven years only three POF cases have been recorded at the KAU Faculty of Dentistry . **The first case** was a 22-year- old Saudi man, reported to the Oral Medicine department in 2007 with a slowly growing painless mass of two years duration. The patient indicated that the lesion was surgically removed seven years ago but came back at the same site five years later. The patient's past medical history was non-contributory. Intraoral examinations revealed a 1.5x1 cm sessile, painless firm, pinkish red dome shaped lesion with some slight surface ulcerations. The lesion located on the buccal Interdental papilla of the right mandibular lateral and cuspid teeth (teeth 42& 43) [Figure 1] **The second case** was a 35-year-old Sudanese woman came to Oral medicine clinic with the chief complaint of a soft tissue mass on the buccal aspect of (teeth 35& 36) of 5month duration. The swelling was 2 x1. 5 cm sessile, mobile, non-tender, and firm in consistency with an irregular surface and bleeding on brushing. The color of the gingiva was pale pink [Figure 2] .This patient also indicated that the lesion was there three years ago when she was pregnant and had been surgically removed. The patient medical history revealed Myasthenia Gravis and thymectomy since 11 years her drug list included pyridostigmine 60 mg and *Azathioprine50 mg* . **Recently** in march 2011 a13-year-old Jordanian healthy boy presented to the clinic with a slow-growing painless mass of one year duration. According to the patient, the lesion appeared 2 years earlier and has been removed in a private clinic, but reappeared again one year later. The lesion is 2.5x1.5 cm firm pedunculated reddish pink with areas of white ulceration, present on the buccal gingiva of (teeth 11, 12, 13) [Figure 3].

The radiographic examinations of all cases were within normal limits, with no findings pertaining to the exophytic lesions. **The differential diagnosis** for the cases included pyogenic granuloma (PG), fibrous hyperplasia, POF, and peripheral giant cell granuloma. Under local anesthesia, the lesions were completely excised with deep scaling of adjacent teeth. The **histopathological examination** of the

lesions revealed the prominent area of highly cellular fibrous connective tissue showing collagen fibers and proliferating plump fibroblasts, and focal areas of trabecular bone lined by osteoblasts [Figure 4]. The diagnosis was POF according to both clinical and histopathological patterns. All the cases were scheduled for follow-up appointments. Up to date the first and third cases showed no evidence of recurrence, while the second case reported second recurrent lesion at the same site within one year period as she got pregnant, and the recurrent lesion was diagnosed as POF.

The results of **vimentin** and **actin** immunohistochemical staining of the cases studied are summarized in table 1. The Immunohistochemistry showed that the POF cells in all POF cases were moderately to strongly positive for **vimentin** and **actin**, indicating fibroblastic-myofibroblastic nature of these lesions. [Figures 5, 6]. However, cases of normal oral mucosa and fibrous hyperplasia showed moderate positive reaction to vimentin in the fibroblast cells, while weak positive reaction to actin was noted only in the cells of the blood vessel's wall. [Figures 7,8]

Table 1: Immunohistochemical staining reaction of vimentin and actin antibodies

Primary antibody	Study cases		
	Normal oral mucosa	Fibrous hyperplasia (Irritational fibroma)	POF cases
Vimentin	++	++	+++
Actin	+	+	++



Fig 1: Clinical picture of the 1st case



Fig 3: Clinical picture of the 3rd case.



Fig 2: Clinical picture of the 2nd case.

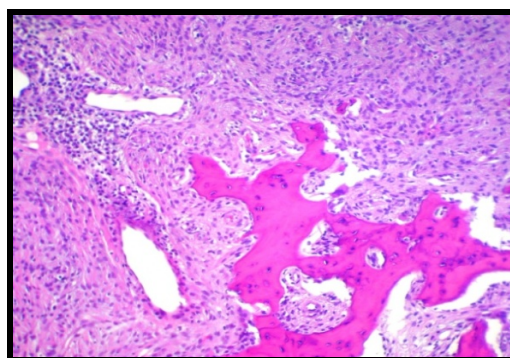


Fig 4: Histopathological view of the second case lesion showing cellular fibrous connective tissue containing bone trabeculae (H&E stained X 200).

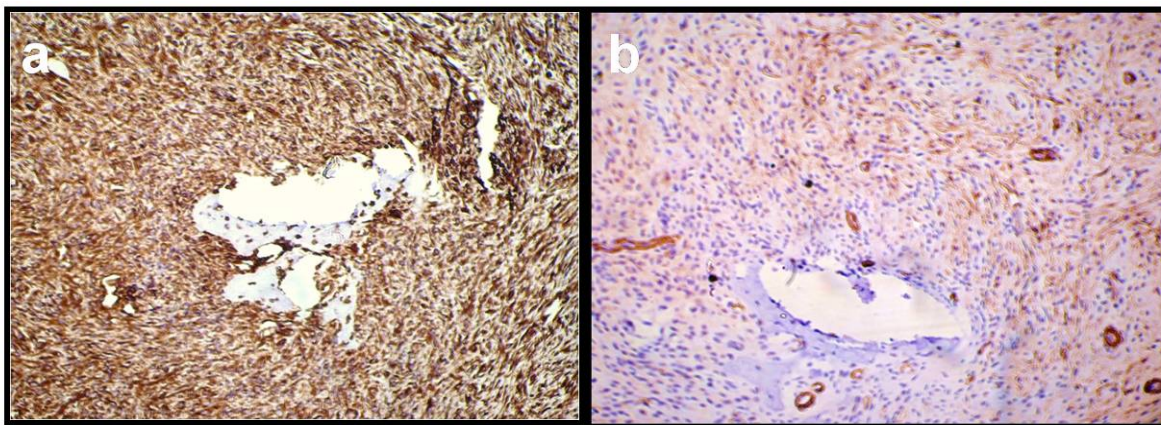


Fig 5: Case 1 of POF showing strong positive staining for vimentin (a) and moderate positive staining for actin (b) (X200).

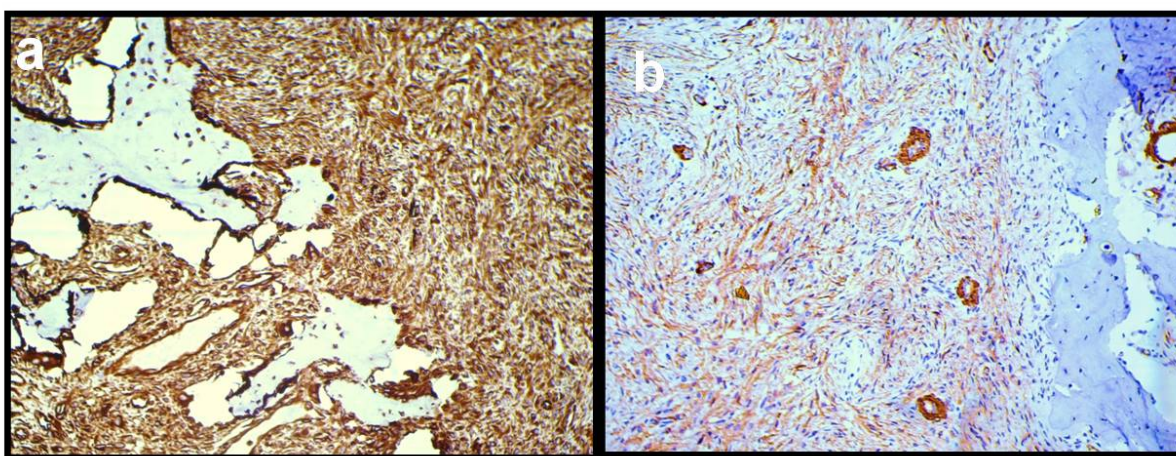


Fig 6: Case 2 of of POF showing strong positive staining for vimentin (a) and moderate positive staining for actin (b) (X200).

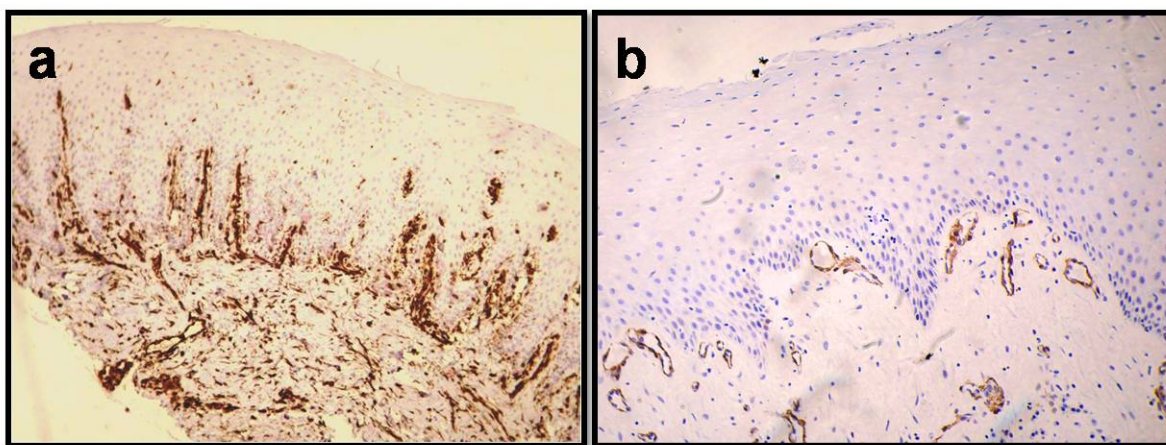


Fig 7: Normal oral mucosa showing moderate positive staining for vimentin (a) and weak positive staining for actin which is localized to the wall of blood vessels (b) (X200).

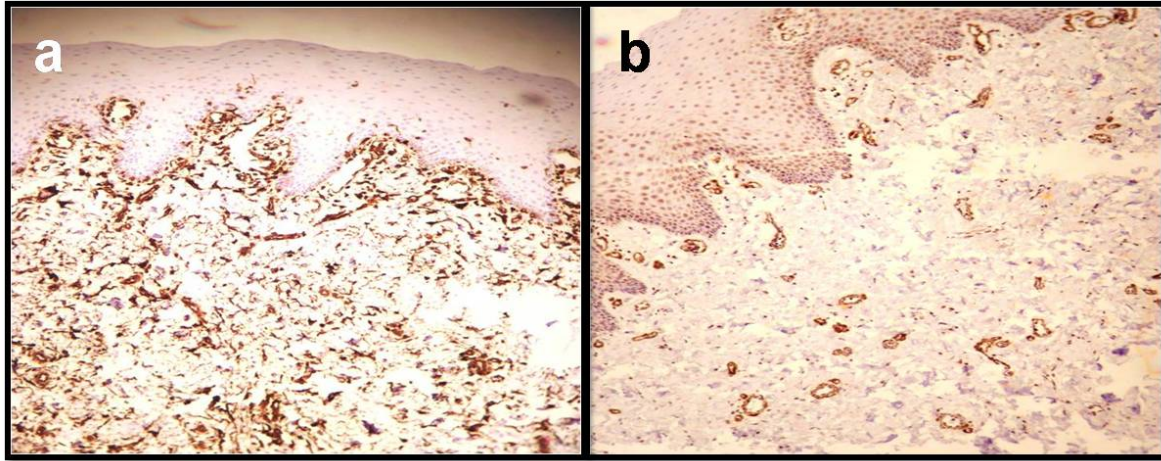


Fig 8. Fibrous hyperplasia showing moderate positive staining for vimentin (a) and weak positive staining for actin which is localized to the wall of blood vessels (b) (X200).

Discussion

The POF is a focal, reactive, non-neoplastic soft tissue growth often occurs exclusively on the gum. Gardner⁽¹³⁾ recommended that the term POF be used to describe non-neoplastic lesion that shows histopathological characteristics of a fibrous cellularized stroma containing a variety of mineralized material, differentiating it from peripheral odontogenic fibroma which is characterized by odontogenic epithelium and dysplastic dentin^(3; 10). Several studies indicated that POF represents a reactive benign lesion and is not the soft tissue counterpart of ossifying fibroma^(13; 14).

POF is a rare lesion comprising nearly 3% of oral lesions biopsies in one study⁽¹⁵⁾, and approximately 1%–2% in other studies^(16; 17). Few studies have been conducted in the Saudi population on oral mucosal lesions. One of those, is an 8-year retrospective study conducted in Riyadh, the capital city of Saudi Arabia at King Saud University the College of Dentistry, using biopsied specimens, found that POFs accounted 5% of all cases of localized inflammatory hyperplasia of the oral cavity⁽¹⁸⁾. This may explain the small number of POF cases reported here in Jeddah, the second city of the country.

Overall, in the present study the clinical findings except for the sex and site correlated well with the reported general characteristics of the POF lesions. By most reports, the majority of the lesions occurred in the second decade of life, in the maxilla, and in both jaws more than 50% occurred in the incisor-cuspid region⁽⁷⁾. Clinically^(1-3; 5), POF manifested as a pedunculated or sessile nodular mass, which usually originates in the interdental papilla. Its color can be pink to red with areas of ulceration. Lesions of POF can show diffuse radiopaque calcification but not all lesions exhibit these

radiographic characteristics, as Shetty *et al.*, indicated that 90% of the POF cases showed no radiographic features⁽⁸⁾ this was in accordance with our finding.

The female to male ratio reported in the literature vary from 2:1 to 3:2⁽⁵⁾. Whereas, in our study it was 2 males:: 1female and was in the mandible more than the maxilla. Interestingly, the female case had the lesions when she became pregnant 3 times, also Marcos *et al.*⁽¹¹⁾, reported 4 female cases of POF, and half were pregnant. While, the relationship between pregnancy and pregnancy tumors which is a unique form of pyogenic granuloma is well established, no one so far touches the relationship between the POF and the pregnancy. Although the clinical characteristics of POF suggest hormonal influence, but Marcos *et al.*, were unable with immunohistochemical study to demonstrate the expression of hormone receptors in the proliferating cellular component⁽¹¹⁾. Further studies are needed to illustrate the hormonal influences on POF.

Though POF is a benign reactive lesion; the recurrent rate is high, as all the cases presented here came with recurrent lesions. A review of the literature showed a recurrence rate of 7-20%⁽¹⁹⁾, some claims the paucity of the access due to the position of the lesion in the interdental area which may contribute to the incomplete removal. Therefore, a deep excision to the base of periosteum & keeping the patient on close regular follow up is mandatory to minimize the recurrence of the lesions.

Although the pathogenesis of POF is uncertain the majority of researchers believed that POF develops from cells of Periodontal ligament (PDL) /periosteum, as hyperplastic growth of tissue^(2; 4; 10). This hypothesis is based on the fact that POFs arise exclusively on the gingiva, the subsequent proximity of the gingiva to the periodontal ligament and the presence of oxytalan fibers within the mineralized

matrix of some lesions ⁽²⁰⁾, and the inverse correlation between age distribution of patients presenting with POF and the number of missing teeth with associated periodontal ligament ⁽⁴⁾. Chronic irritation of the periosteal and periodontal membranes causes metaplasia of the connective tissue and the resultant initiation of formation of bone or dystrophic calcification ^(4; 21). Cundiff ⁽²¹⁾ stated that mineralization is an inherent potential of periodontal ligament and/or periosteum, which would further lead us to dwell on the reasons of their pathogenesis. However, some believed that these lesions were simply a mature variant of pyogenic granuloma, as PG undergoes subsequent fibrous maturation and calcification ⁽²²⁾.

Myofibroblasts are contractile cells that share characteristics of fibroblasts and smooth muscle cells ⁽²³⁾. According to Meng *et al.*, myofibroblasts existed in the PDL ⁽²⁴⁾. The current research has further illustrated the possible origin of POF from cells of PDL, as it investigated the presence and distribution of myofibroblasts in the POF lesions, by means of immunocytochemistry. The results showed that all POF cases were moderately to strongly positive for **vimentin** and **actin**, indicating fibroblastic-myofibroblastic nature of these lesions. Vimentin, desmin and α -SMA are the three filaments most often used to classify myofibroblasts. Among these, α -SMA has been suggested as the most reliable marker of myofibroblast differentiation ⁽²⁵⁾. Garcia de Marcos & co-workers ⁽¹¹⁾ also investigated the presence of myofibroblasts in POF by means of immunocytochemical analysis & showed similar results.

Moreover, in this study we compared the findings in the POF lesions with that in normal gingival tissues as well as localized fibrous hyperplasia lesion (irritational fibroma). Surprisingly, the results revealed the presence of these cells in the POF lesions more than normal gingival tissues & localized fibrous hyperplasia (irritational fibroma). Thus, we can speculate that the POF is a unique lesion originated from the PDL not a mature variant of pyogenic granuloma, as PG is a kind of inflammatory hyperplasia, which includes irritational fibroma, giant cell granuloma, epulis fissuratum, pulp polyp, palatal papillary hyperplasia. However, Filioreanu *et al* reported that, myofibroblasts are present in fibroinflammatory epulis and giant cells peripheral granuloma, by means of immunocytochemical and transmission electron microscopy studies, isolated or grouped, near and toward the inflammatory process ⁽²⁶⁾.

Conclusion

The results represent further evidence of the possible myofibroblastic nature of the POF lesions, and are consistent with a possible origin in the periodontal ligaments however, this is a preliminary study that needs to be repeated with large number of both POF and different forms of inflammatory hyperplasia lesions. The hormonal influence on POF needs further study. Overall, complete removal of the lesions and close postoperative follow-up are important to reduce the possible recurrence.

The authors have no declared financial interests.

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