Original Article

Osteopontin expression and clinicopathologic correlation of oral hyperplastic reactive lesions: An institutional 6-year retrospective study

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Abstract

Background and Objective: Reactive proliferations of oral cavity comprise pyogenic granuloma (PG), fibrous hyperplasia (FH), peripheral ossifying fibroma (POF), and peripheral giant-cell granuloma (PGCG). They often pose diagnostic challenges due to their overlapping clinical and histopathological features. This study was conducted to determine the frequency and clinicopathological correlation of reactive hyperplastic lesions in the oral cavity reported in our institute and compared it with other previous studies. Further evaluation of osteopontin (OPN) expression in normal gingival tissue and different types of focal reactive lesions was also done.

Materials and Methods: Data of all reactive hyperplasias were retrieved, reviewed, and analyzed for age, gender, clinical presentation, and site of location. Presence and distribution of OPN were assessed using immunohistochemistry in these reactive lesions.

Results: Two hundred and forty-eight reactive lesions were comprised of FH (38%), PG (23%), POF (13%), and PGCG (7%). FH was more common in males (55%) whereas other reactive lesions were more in females (68%–73%). The most frequently involved site was gingiva (59%), and most common clinical presentation was sessile growth on gingiva. OPN expression was minimal in normal gingiva. Few cases of FH, PG, and all cases of POF showed positivity for OPN in inflammatory cells, stromal cells, extracellular matrix, and in calcifications.

Conclusion: Reactive hyperplastic lesions of oral cavity are mucosal responses to chronic low-grade irritation caused by plaque, calculus, and any other irritant. It is helpful to know their frequency and presentation as their early identification enables accurate patient evaluation and management.

Keywords: Fibrous hyperplasia, gingiva, osteopontin

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INTRODUCTION

The oral mucosa is subjected to chronic or recurrent irritations such as calculus, ill-fitting dentures, overhanging restorations culminating in a wide spectrum of oral lesions ranging from developmental to inflammatory and reactive to neoplastic diseases.[1,2] Reactive hyperplastic lesions represent

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the most frequently encountered oral mucosal lesions in humans.[3] Kfir *et al*. have classified reactive hyperplastic lesions into pyogenic granuloma (PG), peripheral giant-cell granuloma (PGCG), peripheral ossifying fibroma (POF), and fibrous hyperplasia (FH).[4] Recently, localized juvenile spongiotic gingival hyperplasia has been added to this category by Rossmann.[5]

These reactive oral lesions manifest both clinically and histologically as nonneoplastic nodular swellings. Clinical appearance consists of sessile or pedunculated mass which may be large or small in size indicating a chronic process, in which an exaggerated repair occurs following injury or trauma and usually has no radiographic features.[6] Surgical excision is the treatment of choice and elimination of chronic irritant is mandatory as the persistence of irritation or trauma will cause frequent recurrence.[7]

Earlier, the term "epulis" was used clinically to describe any localized growth on gingiva, but histological examination of such lesions indicates that the majority of them are FH, PG, PGCG, and POF. Their histopathological features are quite distinct but considerable overlap still exists among these lesions.[7] Some authors have postulated that an inflammatory hyperplasia may be the same single lesion which undergoes different stages of maturation and forms a spectrum of reactive lesions.[4,8] Eversole and Rovin speculated that the different histological entities of inflammatory hyperplasia may be due to connective tissue response to varying intensities of mucosal irritation.[9] Persistent reactive lesions for a prolonged period of time sometimes show the formation of calcified structure within connective tissue stroma. The initiating factors influencing the dystrophic calcification or cementum or bone formation in these reactive lesions are poorly understood. Long-standing PG exhibits maturation and dystrophic calcifications that mimic histopathology of POF and even FH for a prolonged period may show calcified structure within the stroma.[10] Hence, the question of whether all these reactive lesions are separate entities or represent different stages in maturation of a single lesion with variable mineralization has not been answered for many years.

Mineralization is usually influenced by collagenous and noncollagenous protein present in the stroma. osteopontin (OPN) is one such noncollagenous highly phosphorylated sialoprotein with extensive calcium-binding potential. It is normally produced in bone, teeth, kidney, epithelial lining tissues and also involved in a number of physiologic and pathologic events such as angiogenesis, apoptosis, inflammation, wound healing, and tumor metastasis.[9] The connective tissue contains certain inhibitory factors which prevent mineralization in normal stroma, but during disease process, these factors are lost. When normal tissue undergoes pathologic changes, OPN is expressed in stromal tissue.[10]

The aim of the present study is to determine the frequency and clinicopathologic features of oral reactive hyperplastic lesions which were reported in the Department of Oral Pathology of a tertiary dental care teaching hospital of Haryana over a period of 6 years and compare this data with similar studies previously reported in literature. An attempt is also made to study the expression of OPN in such reactive lesions

MATERIALS AND METHODS

In this retrospective study, all the existing records in the archives of Department of Oral Pathology, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, were extracted between 2010 and 2015. Patient records were assessed to select those with the diagnosis of reactive hyperplastic lesions as classified by Kfir *et al*. A total of 1380 records evaluated during the respective period, among which 284 of the lesions were reactive hyperplasias. Data including the type of lesion, age, gender, clinical presentation, and the affected site were collected using biopsy requisition forms and their histopathological reports. Comparison of the present study data was done with similar studies previously reported in literature.

For studying OPN expression in reactive hyperplastic lesions, paraffin wax blocks of only those patients who had earlier given consent for carrying out research work on their biopsied material with the diagnosis of FH, PG, and POF were retrieved from departmental archives. Ethical clearance from the Institutional Committee was also taken. The study sample was divided into four groups with ten cases of these lesions in each group. Group A was considered as control group, i.e. normal gingiva. Group 2 includes FH and Group 3 and 4 were consisting of PG and POF, respectively. Care was taken during the selection of cases such that neither of the cases in any group showed surface ulceration microscopically. Immunohistochemical staining of sections cut from formalin-fixed paraffin blocks of each group was done with polyclonal rabbit antihuman OPN antibody (Thermo Scientific, Marietta, Ohio, USA). Slides were stained with appropriate positive and negative controls. The immunostained slides were evaluated by two blinded pathologists independently. All areas of slide in each group were examined and the areas where the intensity was predominant were considered for scoring. These

positive areas were indicated by brown color precipitates, and they were evaluated in stromal cells, extracellular matrix (ECM), calcifications, and inflammatory cells. The fields were scored at ×10 and ×40 with the following scale: 0 (no staining), (1) mild staining, (2) moderate staining, and (3) intense staining. The data were analyzed using Chi-square test to find the difference between the intensity levels among different study groups [Table 1].

RESULTS

From a total of 1380 records evaluated during 6-year period, 284 lesions were reactive hyperplasias. This constituted 20.58% of the total biopsies accessed during this period. The most common lesion was FH with 107 cases (37.7%), followed by 67 cases (23.59%) of PG, 37 cases (13.3%) of POF, and 19 cases (6.7%) of PGCG. Of all the reactive hyperplasias, 102 were males and 146 were females, and the ratio was 1:1.45. The age of patients ranged from 9 to 65 years with a mean age of 37 years. Gingiva was the most common site with 168 cases (59.15%), followed by buccal mucosa with forty cases (14.08%), tongue and vestibule with nine cases (3.17%), lip and alveolar mucosa with seven cases (2.46%), and palate being least involved with five cases (2.1%). Nearly 87% of reactive lesions in the present study showed sessile growth as most common clinical presentation. Table 2 depicts the comparison of clinical data of the present study with previous similar studies.

In the second part of the study, i.e. immunohistochemical evaluation of reactive lesions for OPN expression was examined. All the cases of normal gingiva showed no expression of OPN. FH cases showed OPN positivity in stromal cells and inflammatory cells in all the cases [Figure 1]. PG showed positive OPN expression in ECM, stromal cells adjacent to blood vessels and inflammatory cells [Figure 2]. POF showed remarkable OPN positivity in calcifications resembling bone and cementum, ECM, and few stromal cells [Figures 3 and 4]. Minimal expression was seen in inflammatory cells. The statistical comparison of normal gingiva with FH, PG, and POF was found to be significant ($P < 0.05$). Marked difference was observed in the expression of OPN among ECM and calcifications while comparing FH with PG and FH with POF. On comparing PG with POF, only OPN expression in calcifications was showing highly significant difference ($P < 0.05$). The expression of OPN in the inflammatory cells of FH, PG, and POF showed no significant results [Table 1].

DISCUSSION

Reactive lesions are commonly observed in the oral cavity due to high frequency of tissue injuries and are clinically indistinguishable. A review of 15,783 oral lesions during

Table 1: Comparison of osteopontin expression between control group and study group using Chi-square test

Groups	Inflammatory cells	Stromal cells	Extracellular matrix	Calcifications
1 versus 2	0.000052	0.003	0	0
1 versus 3	0.00026	0.000052	0.001	0
1 versus 4	0.00026	0.000008	0.000008	0.000008
2 versus 3	0.531	0.121	0.001	0
2 versus 4	0.531	0.025	0.000008	0.000008
3 versus 4	0.7	0.305	0.06	0.000008

Table 2: Comparison of clinical data of the present study with previous similar studies

Oral reactive hyperplasias	Past studies	Present study
Frequency	Awange *et al*. - 10.6% Nartey *et al*. - 10.3%	8.7%
Gender (female:male)	Zarei *et al*. - 1:1.8 Aghbali *et al*. - 1:1.4	1.45:1
Common site	Buchner *et al*. and Kfir *et al*. - gingiva Zarei *et al*. and Daley *et al*. - gingiva	Gingiva
Mean age	Esmeili *et al*. - 32.6 years Reichart and Philipsen - 29.16 years Buchner *et al*. - 28.04 years	37 years

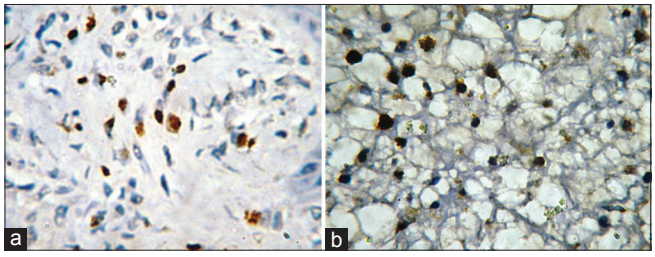

Figure 1: Fibrous hyperplasia showing positive osteopontin expression in (a) stromal cells, (b) Inflammatory cells (H&E, ×40)

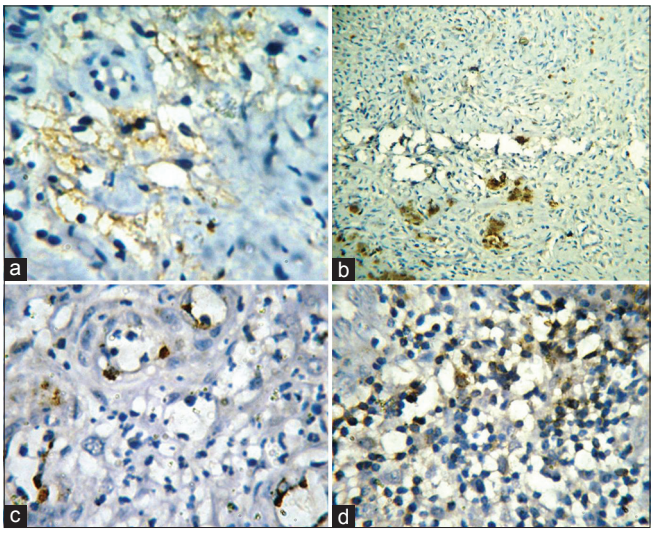

Figure 2: Pyogenic granuloma showing positive osteopontin expression in (a and b) extracellular matrix, (c) stromal cells adjacent to blood vessels, (d) Inflammatory cells (H&E, ×10)

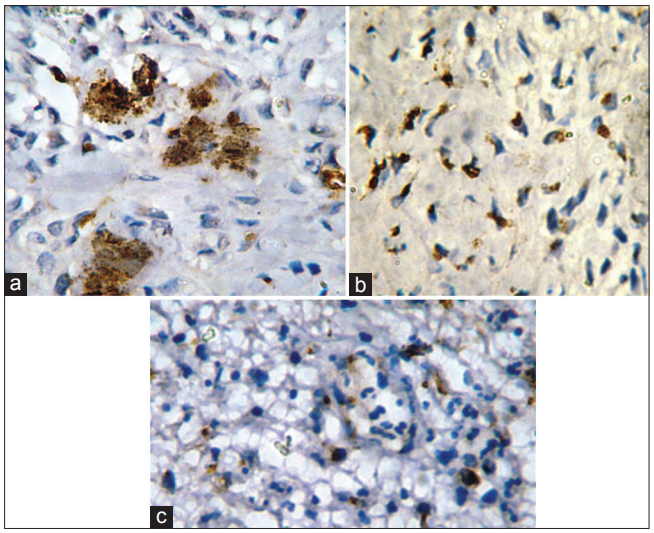

Figure 3: Peripheral ossifying fibroma with positive osteopontin expression in (a) extracellular matrix (H&E, ×4 and H&E, ×10), (b) stromal cells, (c) inflammatory cells (H&E, ×10)

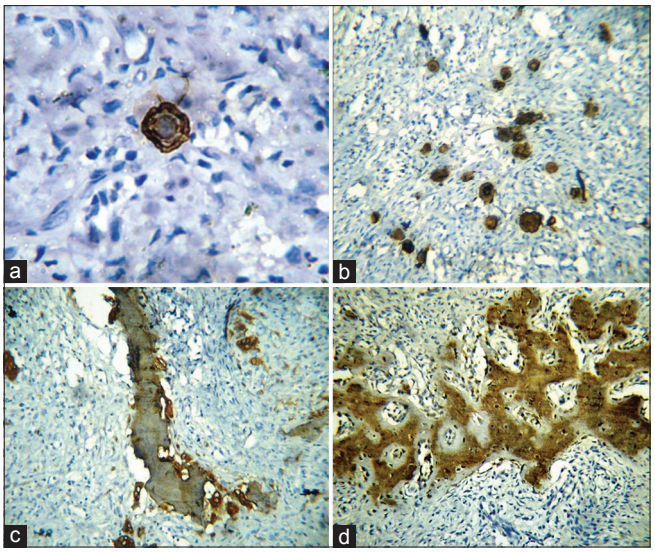

Figure 4: Positive osteopontin expression in calcifications (a and b) resembling cementum (H&E, ×10 and H&E, ×40), (c and d) osteoid (H&E, ×10)

a 17.5 years period by Weir *et al.* in the US found that fibromas, periapical granulomas, mucoceles, and radicular cysts are the most common reactive lesions observed in the oral cavity. It was also found that 77% of lesions observed in oral cavity are reactive in nature.[11,12] Esmeili *et al.* in their review stated that reactive lesions on gingiva rank the second most common among the group of oral reactive lesions.[13] According to Perallas *et al.*, the most reactive gingival lesion is FFH (41%), followed by PG (30%), similar to the findings of the present study (38% and 24%, respectively).[14] The prevalence of gingival reactive lesions in the present study was 20.5% which is higher than the findings of Effiom *et al.*, who reported the prevalence of 5.6% in Nigerian population.[1] A study by Al Rawi in Iraq population showed the prevalence of 15.79%.[15]

Reddy *et al.*[16] observed the prevalence of 12.6% in North Indian population which was comparatively low when compared with the present study (20.5%) and study by Patil *et al.*[17] in Western Indian population (17.4%). The prevalence of reactive lesions of gingiva is reported to be common with peripheral fibroma being the most common category (56%–61%), followed by PG (19%–27%), POF (10%–18%), and PGCG (1.5%–7%) based on over 3000 cases studied in literature.[18] The findings of our study also show the similar prevalence of FH (37.67%), PG (23.59%), POF (13%), and PGCG (6.7%). Other clinical parameters studied in the study were compared with previous similar studies and have been tabulated in Table 2.

Reactive lesions often present diagnostic challenges because of their overlapping and deceptive clinical presentation. Long-standing PG may exhibit dystrophic calcifications which mimic histopathology of POF. Even persistent focal reactive growth of gingiva for a prolonged period may result in the formation of calcified structures within it.[10] The soft tissue possesses certain inhibitory factors that prevent it from undergoing calcification. During disease process, there may be deviation from normal process or elimination of inhibitory factors leading to calcification of soft tissue.[19] When normal tissue undergoes pathologic changes, OPN may be expressed in stromal tissue.[20] This concept is supported by the present study, in which no OPN expression was seen in normal gingiva and other study groups showed variable presentation.

OPN expression was studied in the stromal cells, ECM, inflammatory cells, and calcifications. It was seen in inflammatory cells of FH with variable intensity. In PG, positive expression was seen in inflammatory cells more around the blood vessels and stromal cells. This could be due to inflammation-induced cytokines around blood vessels which stimulate vascular smooth muscles to undergo osteogenic differentiation and thereby producing mild expression of OPN around blood vessels and in stromal cells. However, it is not yet clear whether inflammation-induced osteogenesis or osteoblastic differentiation pertaining to periodontal ligament origin result in such expression.[10] In contrast, POF did not show much expression in inflammatory cells.

ECM of two cases of PG and all cases of POF showed positivity indicating an imbalance in the stroma and initiation of mineralization whereas FH failed to show such ECM expression. All cases of POF showed positive OPN expression

in calcifications resembling bone or cementum. The reason for the presence of calcified structures in POF may be its tissue of origin either from fibrous metaplasia or osteogenic differentiation of cells, in which inflammation can play a role.

OPN expression in the epithelium of gingiva and other study groups was negative confirming that epithelium is not genetically altered in reactive lesions.

Numerous researches have been done in the past to study the role of OPN in calcifications. Increased OPN expression in calcifications of peritoneal wall of patients with continuous ambulatory peritoneal dialysis therapy has been studied by Nakazato *et al.* 2002.[21] Cecilia *et al.* and Hirota *et al* also investigated the role of increased serum OPN levels in severity of atherosclerosis.[22,23] Ono *et al.* in their study showed that OPN deficiency enhances parathyroid hormone-related peptide receptor (PPR) signaling-induced alteration in tooth formation and odontoblastic morphology.[24]

Similar to the present study, an attempt was made by Elanagai *et al.* in 2015 to study OPN expression in reactive lesions of gingival.[10] Their results suggest that there is osteoblastic differentiation of stromal cells in focal reactive lesions of gingiva. Our study is a second attempt after Elangai *et al.*[10] to examine the overlapping reactive lesions immunohistochemically. The role of OPN in calcinosis is still controversial; it may contribute to crystal growth, stabilization, rather than to nucleation of hydroxyapatite, in the presence of ECM.

CONCLUSION

Numerous studies in the literature have been done on OPN levels in serum, saliva and gingival crevicular fluid of patients for various hypotheses. This study appears to be second attempt to read OPN in connective tissue stroma of oral lesions. Again we emphasize on the issue that whether these lesions are separate entities or different phases during maturation of single entity, more studies need to be carried out using specific markers for osteoblast, cementoblast, and in development of ossification.

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Conflicts of interest
There are no conflicts of interest.

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