So-called “Denture fibroma”: A Histopathological and Immunohistochemical Study

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Abstract: The purpose of this study was to investigate the histopathological and immunohistochemical characteristics of so-called “denture fibroma” and related stromal reaction. The tissues were histologically classified into inflammatory granulation type, matured fibrous type and myxoid type. Tenascin, LOX, fibrinogen and COX were strongly detected in inflammatory granulation type whereas a weak expression was detected in matured fibrous type. LOX was remarkably expressed by spindle shaped cells/fibroblastic cells and endothelial cells while tenascin was relatively expressed by monocytes in myxoid type. Despite strong expressions of tenascin, fibrinogen, COX-2 and LOX, their expressions decreased towards the deep part of the connective tissue. The greatest number of vessels was obtained in inflammatory granulation type and the least number of vessels was obtained in myxoid type which might contribute to hypoxic condition.

The results suggest that the so-called “denture fibroma” is a consequence of acute inflammatory reaction leading to the formation of inflammatory granulation followed by the state of fibrosis which gradually was replaced by matured collagen fibers. Furthermore, myxoid degeneration occurred when blood vessels decreased which lead to hypoxic condition.

Key Words: Denture fibroma, Irritation fibroma, Immunohistochemical study

Introduction

The so-called “denture fibroma”, one of the oral irritation fibromas, is a benign reactive tumor of fibrous connective tissue formed in response to ill-fitting complete or removable partial dentures. It is relatively a common lesion with an incidence rate of 4.9 to 18.0% among denture wearers. Predilections in the anterior region, among female patients, complete denture wearer, and in those patients where the denture has been worn for more than 10 years have been described in previous epidemiological studies ¹-¹⁵).

Histopathologically, irritation fibroma is characterized by having a dome shape, lined by keratinized stratified squamous epithelium. The supporting connective tissue is composed of dense bundles of collagen fibers with spindle- or fibroblast-like cells, relatively few blood vessels and inflammatory cells ¹⁶). Various terms have been used denoting its microscopic features including “denture hyperplasia, denture-induced fibrous hyperplasia, denture-induced inflammatory fibrous hyperplasia, denture injury tumor and flabby gum ²-⁴, ⁶-⁹, ¹⁷-¹⁹). Oral irritation fibroma has been recognized as a hyperplasia of fibrous tissue and not a true tumor. However, few reports have observed factors in connection with hyperplastic connective tissue formation.

The purpose of the study was to investigate the histopathological and immunohistochemical characteristics of so-called “denture fibroma” and related stromal reaction.

Materials and methods

1. Subjects

A total of 12 cases of so-called “denture fibroma” diagnosed histopathologically by 2 oral pathologists from 2001 to 2011 at the Department of Diagnostic Pathology, Nihon University School of Dentistry at Matsudo Hospital were used in this study. The patients consisted of 3 males and 9 females with age ranging from 45 to 82 years with an average of 70 ± 11 years. Six of the lesions were located in the maxilla/mandible and 6 were in the anterior/posterior regions. Excised specimens were fixed in 10% neutral formalin solution and then embedded in paraffin blocks according
to standard method. Serial sections of 4 μm thickness were prepared from paraffin blocks for histopathological and immunohistochemical examination.

The patient’s agreement, privacy, diagnostic outcome and management were considered in this study. Informed consent was obtained from all patients before retrieving the pathological specimens.

2. Light microscopy and histochemical staining

Histological examination was done in all cases using hematoxylin and eosin (H.E.) stains. Immunohistochemical studies were conducted using 10% neutral formalin solution-fixed, paraffin-embedded tissues. Sections were deparaffinized in xylene and hydrated in graded ethanol solution. EnVision+ Polymer System (Dako Glostrup, Denmark) was used for antigen detection. Primary antibodies used were directed against the following antigens: tenascin-c (Tenascin, DB7, 1:100; BIOHIT); lysyl oxidase (LOX, 1:100; Santa Cruz); fibrinogen (fibrinogen, 1:500; Dako) and COX-2 (COX-2, 1:100; Dako). Antigen retrieval was performed in a pressure cooker with citrate buffer solution with a pH of 6.0 for LOX and fibrinogen and a pH of 9.0 for tenascin and COX-2. Secondary antibody reaction was carried out using EnVision+ Polymer System (Dako Glostrup, Denmark). Antigenic reactions were detected using 3, 3′-dianibobenzidine tetrahydrochloride (DAB) and then counterstained with Mayer’s hematoxylin. Specimens of cervical squamous cell carcinoma (LOX and tenascin) and inflammatory granulation tissue (fibrinogen and COX-2) were used as positive controls. For evaluation of immunohistochemical staining technique, negative controls were used where in mouse and rabbit universal negative controls (Dako Glostrup, Denmark) were used instead of primary antibodies.

Figures 1-4  Histopathological and immunohistochemical findings in inflammatory granulation type (1), matured fibrous type (2), myxoid type (3), and subepithelial region of matured fibrous type (4)

a: Histopathological appearances of connective tissue (H.E. stain, x10)
b: COX-2 showed positive reaction in inflammatory granulation type (Fig.1-b) and monocytes of myxoid type (Fig.4-b) (x10)
c: Fibrinogen showed remarkably positive reaction in the inflammatory granulation type (Fig.1-c) and subepithelial lesion of the mixed type (Fig.4-c) (x10)
d: Positive finding of Tenascin was apparent in the inflammatory granulation type (Fig.1-d), and diffusely positive in the myxoid type (Fig.3-d) (x10)
e: Positive findings for LOX was clearly observed in the inflammatory granulation type (Fig.1-e) and myxoid type (Fig.3-e) (x10)
1. **Histopathological analysis**

Microscopically, the so-called “denture fibroma” appeared as a nodular mass of fibrous connective tissue covered with stratified epithelium. The connective tissue was either dense or less collagenized. The so-called “denture fibromas” were classified into inflammatory granulation type, matured fibrous type and myxoid type.

The combination of inflammatory granulation and matured fibrous types was also observed. The inflammatory type consisted of scattered inflammatory cells with slight to moderate vascular proliferations. The formation of lymphoid follicle by inflammatory cell infiltrates was also observed (Fig.1-a). The matured fibrous type mainly consisted of dense eosinophilic collagenous stroma with few to moderate number of spindle cells. Few inflammatory cell infiltrates was observed lining the blood vessels with fibrous bundles (Fig.2-a). The myxoid type was mainly composed of prominent loose myxoid stroma with relatively large spindle cells that were scattered. Dilated capillaries were likewise observed (Fig.3-a). Inflammatory reaction in the underlying connective tissue was noted in all types of so-called “denture fibromas” (Fig.4-a). The inflammatory granulation type had the most number of blood vessels (5 blood vessels per filled at high magnification, p<0.01) followed by the mature fibrous type and the myxoid type having the least number of blood vessels (Fig.5).

2. **Immunohistochemical findings**

Immunohistochemical findings were summarized in Table 1. A strong reaction to COX-2 was observed only in inflammatory granulation type localized in undifferentiated mesenchymal cells, spindle shaped cells, endothelial cells and monocytes. A weak expression was observed in mature fibrous type localized in spindle-shaped cells but moderate expression in scattered monocytes. Relatively strong positive reaction in monocytes was detected in myxoid type.

Strong expression of fibrinogen localized in spindle shaped cells/fibroblastic cells were diffusely detected in inflammatory granulation type. Weak to moderate expression localized in spindle shaped cells/fibroblastic cells were detected in matured fibrous type. A sparse reaction localized in spindle shaped cells/fibroblastic cells was detected in myxoid type.

Tenascin was detected strongly in inflammatory granulation type (Fig.1-b), a weak but scattered positive reaction was detected in matured fibrous type, and a diffusely positive reaction was noted in myxoid type (Figs.2-b, c).

Undifferentiated mesenchymal cells around the capillaries were strongly positive to LOX in inflammatory granulation type. Weak but scattered positive reaction of spindle shaped cells/fibroblastic cells were detected in matured fibrous type (Fig.2-b). A remarkable positive reaction in spindle shaped cells/fibroblastic cells and endothelial cells were detected in myxoid type (Fig.3-b). In spite of the strong expressions of tenascin, fibrinogen, COX-2 and LOX, a decreased in the expression was observed in the deeper part of the connective tissue (Figs.4-b-e).

**Discussion**

Reactive hyperplasia caused by chronic injury to tissue in contact with the denture border is the most common type of exophytic lesion among oral inflammatory/reactive lesions 1). Different clinical terms have been used to describe reactive hyperplasia caused by denture on the basis of microscopic features and oral pathologist’s view. Review of literatures revealed denture hyperplasia, denture induced hyperplasia and denture induced fibrous hyperplasia are the terms most commonly used. In this study, we described this denture induced tumor-like lesion as so-called “denture fibroma”.

Few reports concerning the histochemical and immunohistochemical

<table>
<thead>
<tr>
<th>Histopathological type</th>
<th>No.*</th>
<th>LOX</th>
<th>Tenascin</th>
<th>Fibrinogen</th>
<th>COX-2</th>
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<tbody>
<tr>
<td>Inflammatory granulation type</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Matured fibrous type</td>
<td>5</td>
<td></td>
<td>±</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Myxoid type</td>
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<td></td>
<td></td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

++: strong positive, +: moderate positive, ±: weak positive, -: negative

No*: Number of cases
studies of so-called “denture fibroma have been published. Epithelial proliferation and the presence of elastin fiber were identified in so-called “denture fibroma” in a previous report. This lesion is not a true neoplasm and the lesion appears as an exaggeration and overgrowth of restoration after an inflammatory phase associated with cell infiltration and cytokine production induced by chronic stimulus. Histopathologically, the combination of fibrous and inflammatory granulation was predominant in previous report.

COX-2 could be induced by a variety of pro-inflammatory agents such as cytokines, bacterial endotoxin and tumor necrosis factor which was classified as an early intermediate response gene. There are at least two isozymes of cyclooxygenase namely COX-1 and COX-2. The latter functions together with prostaglandin E2 produced by fibroblasts. Moreover, COX-2 was described to control many aspects of inflammatory reactions and activation of inflammatory cells. Immunohistochemical localization of COX-2 appeared in wound margin in particular.

In the present study, only inflammatory granulation type showed apparently strong reaction to COX-2 localized in undifferentiated mesenchymal cells, spindle shaped cells, endothelial cells and monocytes. Strong COX-2 expression in fibroblasts could stimulate their own activation, migration, and/ or proliferation. Therefore, it was suggested that inflammatory granulation tissue formation was caused by fibroblastic stimulation accelerated by COX-2 in ill-fitting denture. Regarding the relevance of COX-2 and fibrosis, decreased COX-2 activation and PGE2 levels during tissue repair were associated with increased fibrosis and poor repair outcomes in the lung. From the above results, in matured fibrous type, the transition from inflammatory to fibrous tissue type was thought to have been caused by decreased COX-2 expression.

Conversely, the weak expression in spindle shaped cells and moderate expression in monocytes were diffusely detected in mature fibrous type. Increased COX-2 in human monocytes was caused hypoxia. Herein, hypoxia was expected as one factor in myxoid degeneration.

The role of fibrinogen, one of the acute-phase proteins, evolved from a marker of vascular rupture of some signaling molecule for fibrosis, protection from infection and extensive inflammation. The early phases of wound healing involve the formation of extracellular matrices containing fibrin, fibrinogen, and fibronectin. Recent studies also suggested that excessive fibrinogen production might play a role in upregulating host immune responses. In the present study, strong expression of fibrinogen in inflammatory granulation type was considered an early event in organizing inflammatory granulation tissue.

Weak positive reaction to fibrinogen was observed in matured fibrous type in this study. Matured fibrous type was considered to be composed of stable fibrin network and early reaction by fibrinogen. Fibrosis is characterized by excessive accumulation of collagen and other extracellular matrices. In this hyperplastic lesion, the amount of matured collagen fibers increased with both patient’s age and duration of the lesions. Therefore, fibrosis was considered in the deeper part of the connective tissue while continuous chronic inflammatory reaction occurred immediately below the epithelium in so-called “denture fibroma” which coincided with the immunohistochemical findings.

Tenascin is a large glycoprotein component of extracellular matrices with a molecular weight of 190-250 kD. Inflammatory cytokines can potently upregulate tenascin expression secreted by mesenchymal cells during inflammatory reaction. Tenascin was strongly expressed in inflammatory granulation type and diffusely expressed in myxoid type. Previous studies have shown the increased accumulation of tenascin at the subepithelial zone during inflammatory reaction in the oral mucosa. In myxoid type, the relationship between myxoid degeneration and hypoxia was presumably suggested by the appearance of COX-2 in monocytes immunohistochemically. Previous study mentioned that inflammatory cells and stromal fibroblasts were stimulated by hypoxia to express high levels of tenascin.

Lysyl oxidase gene family comprises five members acting as extracellular modulating enzymes. The first identified and the most studied isoform of this family is LOX. It is a copper-dependent amine oxidase expressed and secreted by fibrogenic cells that initiates the covalent cross-linking of collagens and elastin in extracellular matrices. Moreover, LOX promoted remodeling of extracellular matrices under hypoxia and regulated extracellular matrices in oral submucous fibrosis.

From pathomorphological analysis, the highest number of vessels was observed in inflammatory granulation type. The least number of vessels in same area was observed in myxoid type which might easily lead to hypoxic condition.

Consequently, the so-called “denture fibroma” was a result of inflammatory granulation tissue formation which previously underwent acute inflammatory reaction followed by fibrosis which was gradually replaced by matured collagen fibers. Furthermore, the decrease in blood vessels led to an inclination towards hypoxic condition which occurred in the myxoid degeneration type.

References


