Oncocytic metaplasia in inflammatory fibrous hyperplasia: Histopathological and immunohistochemical analysis

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Abstract

Oncocytic metaplasia (OM) is not a well-known feature in inflammatory fibrous hyperplasia (IFH) lesions, although it may be common, as proposed in our previous study about this lesion. In the present paper, we assessed the histopathological and immunohistochemical features of 18 cases of IFH containing OM areas. All the samples were examined on haematoxylin and eosin stained sections and cytokeratins (AE1/AE3, 34ßE12, CK5, CK7, CK8, CK13, CK14 and CK19), CD15, CD20, CD68, CD45Ro, and LCA primary antibodies were used. The vast majority of IFH occurred in women (n=14) and the most common site of presentation was the buccal vestibule. Oncocytic and salivary duct cells showed uniform immunoreactivity for AE1/AE3, CK7, CK8 and CK19. CD45Ro+ T-lymphocytes were the most common inflammatory cells surrounding the OM areas followed by CD20+ B-lymphocytes. These findings suggest that oncocytic cells present in IFH might develop from salivary duct epithelium, and T-lymphocytes might play an important role in its etiopathogenesis.

Key words: *Oncocytic metaplasia, inflammatory fibrous hyperplasia, immunohistochemical, histopathology, T-lymphocytes.*

Introduction

Inflammatory fibrous hyperplasia (IFH), also called denture-induced fibrous hyperplasia and epulis fissuratum, is the most common lesion of the oral mucosa, usually associated to an ill-fitting denture (1). This lesion is easily recognised and in some cases distinct microscopic variations such as osseous, oncocytic and squamous metaplasia may be found. It is caused by chronic trauma generated by ill-fitting denture, where denture edges contact the adjacent tissue (2).

Oncocytic metaplasia (OM) is a well-documented feature in the major salivary glands, which mainly affects elderly patients (3). OM has also been reported in minor salivary glands, particularly in the larynx and pharynx (3-5). However, its occurrence in IFH is poorly documented (6, 7). Oncocytic cells may develop from duct cells but its source is still debatable (3).

The aim of this study was to determine the histopathological and immunohistochemical (IHC) features of OM areas in IFH, in order to better understand their etiopathogenesis and to characterize the cellular inflammatory infiltrate present around these areas.

Patients and Methods

- *Clinical data*

Archived formalin-fixed, paraffin-embedded tissue blocks of 18 cases of IFH with OM areas were obtained from the Oral Pathology Section, Piracicaba Dental School, UNICAMP-Brazil. Clinical data were collected from the clinical charts. All the samples were examined on haematoxylin and eosin (H&E) stained sections. The study was approved by the Piracicaba Dental School Research Ethics Committee.

In a total of eighteen patients, fourteen were female and four male, with a mean age of 64.3± 9.3 years (range from 47 to 82 years). IFHs were present in the oral cavity from several months to 5 years before the surgical excision. Clinically, all lesions presented as submucosal nodules (average size: 0.5 to 6 cm in the maximum diameter), and the most common site of presentation was the vestibular sulcus (9 cases), followed by the alveolar ridge (5 cases), buccal mucosa (2 cases), and floor of the mouth (2 cases) (Fig. 1). All cases were treated by complete surgical excision and no recurrences have been detected.

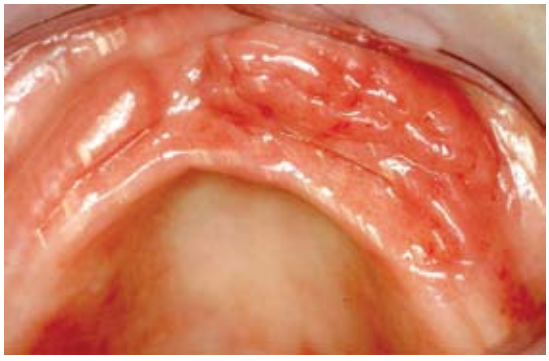


Fig. 1. Nodular mass (IFH) located in the anterior maxillary vestibular sulcus.

- *IHC and histomorphometric analysis*

Immunostaining was performed on 3µm paraffin sections and mounted onto coated-xylan slides. Sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by quenching the sections in 3% hydrogen peroxide. The antigen retrieval was performed using citrate-phosphate buffer pH6 in a microwave oven. Next, the samples were incubated with the primary antibody as described on the Table 1, using appropriate positive and negative controls, and biotynilated secondary antibodies conjugated with streptavidin-biotin-peroxidase (StreptABComplex/HRP Duet, Mouse/Rabbit, Dako A/S, Denmark), which were revealed using the diaminobenzidine chromogen solution, and counterstained with Carazzi's haematoxylin. The immunoreactivity was classified as positive or negative.

The OM areas and residual minor salivary gland tissues were assessed to determine the cytokeratins (CK) profile. The immunoreactivity of inflammatory cells present around the OM areas was assessed by counting of positive cells in 10 fields of 21 208.57 µm2 (System KS-400 2.1 version), establishing, if necessary, an average between them.

- *Statistical analysis*

Relationships between data of inflammatory cells surrounding the OM areas were analyzed using a two-tailed T test. A p value below 0.05 was considered statistically significant.

Results

- *Histopathological findings*

All the cases showed focal (n=6) or multiple (n=12)

Table 1. Primary antibodies, sources and protocols used in the present study.

Antibody	Source/Clone	Dilution	Antigen retrieval
Broad spectrum CK	Dako[1], AE1/AE3	1:500	Mw
CK5	Novocastra[2], XM26	1:400	Mw
CK7	Dako, OV-TL12/30	1:400	Mw
CK8	Dako, 35βH11	1:200	Mw
CK13	Novocastra, KS-1AE	1:400	Mw
CK14	Novocastra, NCL-L-LL002	1:200	Mw
CK19	Dako, RCK108	1:400	Mw
HMW-CK	Dako, 34βE12	1:200	Mw
CD15	Dako, C3D-1	1:200	Mw
CD20 (B-cells)	Dako, L26	1:10000	Mw
CD68 (macrophages)	Dako, PG-M1	1:400	Mw
CD45RO (T cells)	Dako, UCHL1	1:200	Mw
LCA	Dako, PD7/26/16E2B11	1:2000	Mw

1Dako A/S, Denmark; 2Novocastra Laboratories, UK; CK: cytokeratins; Mw: microwave; LCA: leukocyte common antigen; HMW: high molecular weight.

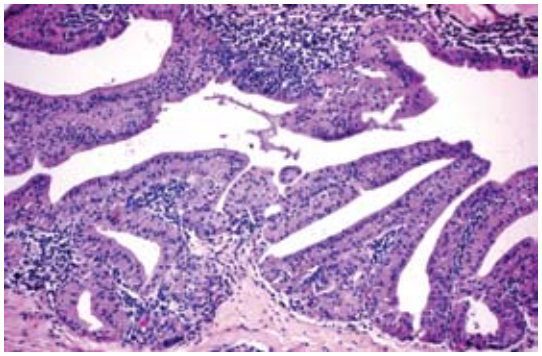


Fig. 2. OM area displaying intraluminal papillary projections associated to chronic inflammatory infiltrate (H&E, OM X10).

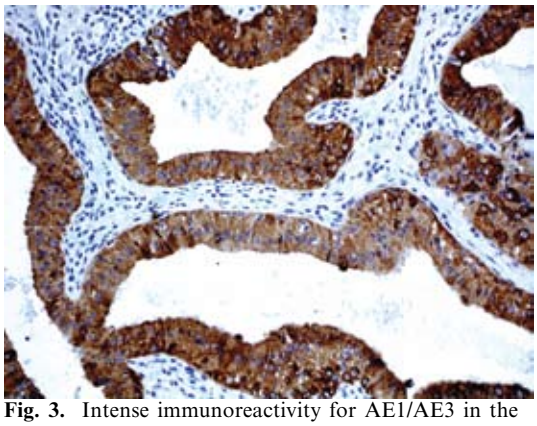


Fig. 3. Intense immunoreactivity for AE1/AE3 in the luminal and non-luminal cells (OM X20).

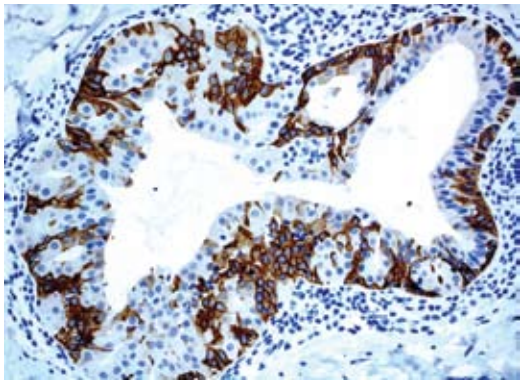
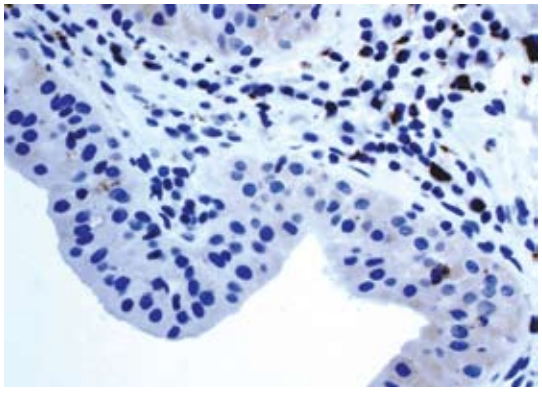


Fig. 4. Basal cells located at the periphery of the OM area showing intense reactivity for CK5 (OM X20).



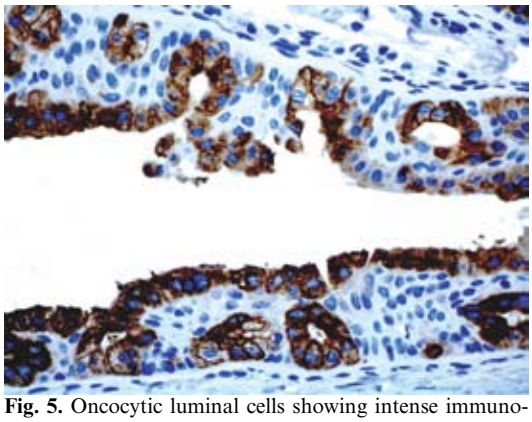


Fig. 5. Oncocytic luminal cells showing intense immunoreactivity for CK7 (OM X40).

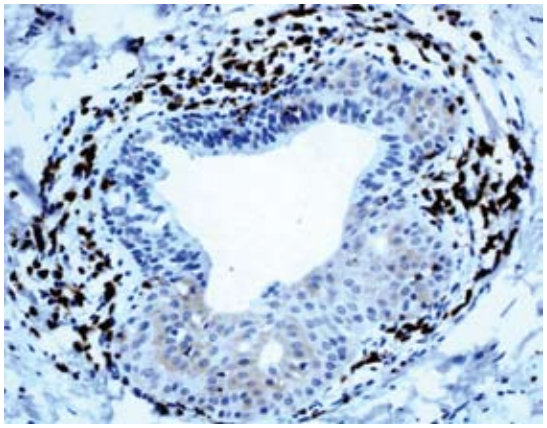


Fig. 6. OM area showing cellular inflammatory infiltrate richly composed by CD45Ro+ T-lymphocytes (OM X20).

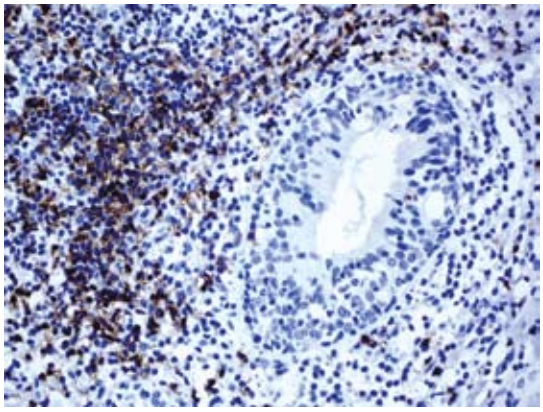


Fig. 7. Nodular aggregate of CD20+ B-lymphocytes around OM area (OM X20).

Fig. 8. Scarce CD68+ macrophages adjacent to OM area (OM X40).

OM areas. In these latter, it was detected 2 to 14 OM areas (mean of 4,8 per case), and intraluminal papillary projections surrounded or not by cellular inflammatory infiltrate (Fig. 2), which simulated a cystadenoma (n=3) or Warthin´s tumour (n=2), were found. Fourteen cases showed well-defined double-layered oncocytic epithelium areas. Moreover, inflammatory cells and scarce secondary lymphoid follicles (1 to 5) were seen adjacent to the surface epithelium in 7 cases.

- *IHC study*

CK immunoreactivity in the OM areas and adjacent normal minor salivary glands.

AE1/AE3 was uniformly expressed in the luminal, basal and myoepithelial cells of the normal minor salivary glands, and in the luminal and basal cells of OM areas (Fig. 3). The myoepithelial and basal cells of the striated and excretory ducts of normal minor salivary glands and the basal cells of OM areas were positive for CK34ßE12, CK5, and CK14 (Fig. 4). The luminal cells of the striated and excretory ducts of normal minor salivary glands and OM areas were positive for CK7, CK8, and CK19 (Fig. 5). The CK13 was uniformly negative in all normal minor salivary glands and OM areas. All mucous acini were negative for these markers.

- *Inflammatory infiltrate characterization around OM areas*

Intense immunoreactivity for leukocyte common antigen (LCA) was seen in the cellular inflammatory infiltrate encircling the OM areas and adjacent to the surface epithelium. CD45Ro+ T-lymphocytes were the most common cells (Fig. 6), and only 4 cases showed predominance of CD20+ B-lymphocytes. Seven cases displayed secondary lymphoid follicles (1 to 5) adjacent to the surface epithelium (Fig. 7). CD68 immunoreactivity was found adjacent to the surface epithelium and microulcerated areas (Fig. 8). CD15+ granulocytic cells were sporadically detected on both microulcerated and perivascular areas.

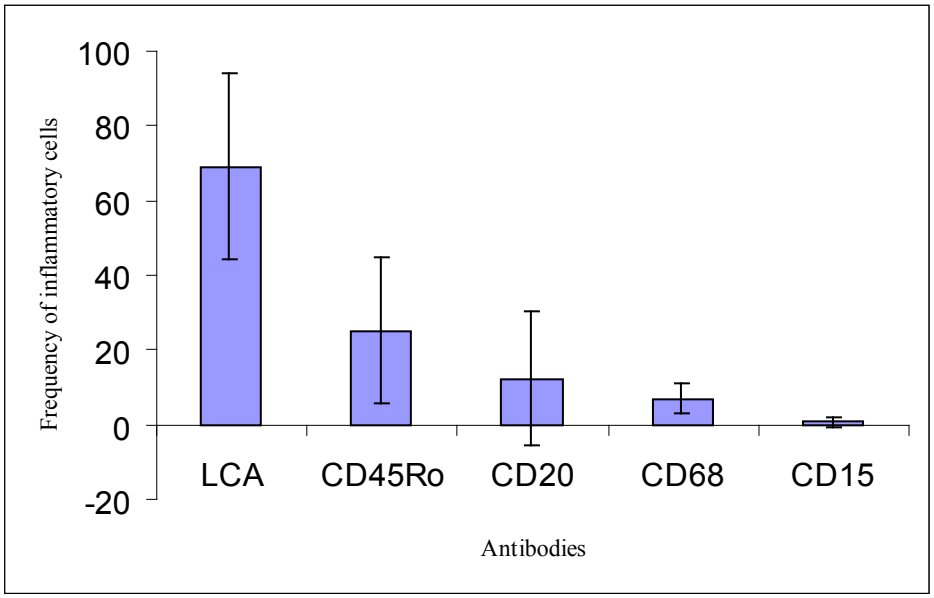
The number of CD45Ro+ T-lymphocytes was significantly higher than CD20+ B-lymphocytes ($p<0.041$), CD68+ macrophages ($p<0.0005$), and CD15+ granulocytic cells ($p<0.00001$). In addition, the number of CD20+ B-lymphocytes was significantly greater than CD68+ macrophages ($p=0.1876$) and CD15+ granulocytic cells ($p=0.0135$) (Fig. 9).

Discussion

IFH is caused by chronic trauma produced by partial or total ill-fitting denture (2, 8). It affects predominantly women (5:1) in the fifth and sixth decades of life. In our study, IFH with OM areas occurred mainly in women (3,5:1) in the sixty decade of life, suggesting that the age and use of ill-fitting denture might be important clinical factors to its occurrence.

Oncocytes are characterized histologically by the presence of finely granular eosinophilic cytoplasm resulting from an increased number of mitochondria. As oncocytes divide, their pathologic metabolic activity and mitochondrial phenotype are passed on to their progeny. These cells produce tissue lesions that range from focal OM and hyperplasias to benign and malignant neoplasms (9). OM is characterized by the replacement of ducts and acini of seromucinous glands by cells with abundant brightly eosinophilic granular cytoplasm (10), which can be seen in normal and pathological tissues, particularly in parathyroid, salivary,

Fig. 9. Histogram showing the immunoreactivity of inflammatory cells (%) surrounding the OM areas (n=18). The CD45Ro+ T-lymphocytes were the most prevalent cells, followed by CD20+ B-lymphocytes, CD68+ macrophages and CD15+ granulocytic cells.



lachrymal, and mucous glands of the upper aerodigestive tract (11). Oncocytic, squamous, and osseous metaplasia can be found in IFH, and OM is frequently and directly associated with chronic inflammatory infiltrate (7). Our results showed that oncocytic changes in IFH might be related to inflammation or increased cellular metabolism as well as a degenerative phenomenon caused by cellular aging (11). We cannot discard that OM could be the response to partial duct obstruction of minor salivary glands caused by constant pressure from overextended borders denture (12). However, their exact significance and the biological function are still unknown (10).

The intercalated, striated, and excretory ducts and acinic cells of the normal salivary glands are positive for CK7, CK8, CK18 and CK19 (13). The myoepithelial cells are CK14 positive, while that the basal cells located in the striated and excretory ducts are reactive for CK7, CK14 and CK19 (14,15). The origin of oncocytic cells remains controversial. However, most evidences favour a duct cell origin, but acinic cell origin has also been proposed (3). The IHC profile of the luminal and basal layers of Warthin's tumour showed similar immunoreactivity to striated duct and basal epithelial cells of the parotid gland, respectively (14,16). On the other hand, Terada and Taniguchi (17), assessing an intraductal oncocytic papillary carcinoma of the liver, showed that the luminal oncocytic cells were immunoreactive for CK7, CK18 and CK19. Recently, we reported a case of denture hyperplasia with areas simulating oral inverted ductal papilloma, and observed intense immunoreactivity for CK7 and CK14 in the luminal and basal cells, respectively (18). Take together, we can hypothesize that oncocytic cells seen in IFH could derive from salivary duct cells as result of the aggression caused by lymphocytic infiltrate, as well as due to the constant pressure by overextended denture borders causing partial salivary duct obstruction. Our IHC findings indicate a duct cell origin for these cells, since both cells exhibit similar profile for CK7, CK8 and CK19.

CK expression is almost identical in serous and mucous acini (19). As differentiation proceeds, secretory cells gradually develop extensive rough endoplasmic reticulum then accumulate secretory granules that may lead to the displacement of the intermediate filaments to the cell periphery. Thus, the expression of intermediate filaments does not depend on the type of secretion granules but may rather be related to the number of granules storage (13). These features might explain the lack of immunoreactivity for CK seen in all the mucous acini of our cases.

van Loon et al. (20) found a high proportion of CD8+ T-lymphocytes in the normal human oral mucosa and skin, whereas those B-lymphocytes were scarce or rare. In our study, the vast majority of OM areas were directly associated with lymphocytic infiltrates and CD45Ro+ T-lymphocytes were the prevalent cells with occasional predominance of CD20+ B-lymphocytes. Such association favours that OM areas are reactive lesions, because of response to the lymphocytes while trying to increase production of high-energy phosphate (9).

In summary, we described the histopathological and IHC features of OM areas in IFH. Our results indicate that the oncocytic cells seen in the OM areas might develop from salivary duct epithelium and T-lymphocytes might play an important role in their etiopathogenesis. Further studies are needed to confirm our results.

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