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MARKERS FOR MYOFIBROBLASTS IN INFLAMMATORY GUMS PSEUDOTUMORS

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ABSTRACT. We have investigated the presence of α-actin and vimentin in biological samples harvested from two patients one diagnosed with fibro-inflammatory epulis and one diagnosed peripheral giant cell granuloma. In the fibro-inflammatory epulis, labelling for vimentin was important for the connective cells in the deep chorion but positive vimentin cells were observed also near chronic inflammatory reaction. Actin immunolabelling was positive for the myofibroblasts in the deep chorion, in the smooth muscle cells in the mid-vessels walls or in the myofibroblasts near the imflammatory process.

Keywords: α-actin, vimentin, myofibroblast, imunolabelling, epulis

INTRODUCTION

In addition to the peripheral giant cell granuloma, mesenchymal cells of the periodontal ligament are capable of producing another unique inflammatory hyperplasia, the peripheral ossifying fibroma, also referred to as the peripheral cementifying fibroma, depending on whether or not bone or cementum is seen microscopically. The pluripotential cells of the ligament have the apparent ability to transform or metaplastically alter into osteoblasts, cementoblasts or fibroblasts. This represent a reactive lesion, not the peripheral counterpart of the intraosseous neoplasm called central cemento-ossifying fibroma. Odontogenic lesions of the gingiva, moreover, may produce various calcified materials.

Myofibroblasts are modified fibroblasts (Powell et al., 1999) which were first described in granulation tissue and have been identified in normal tissues and as the predominant cell in certain reactive lesions (Genco, 1996). In granulation tissue they are probably derived from local fibroblasts, in response to mechanical stress, and their functions, appearance and immunoprofile vary in relation to the phase of activity (Rees, 2000). This is reflected in the variable morphology of reactive lesions such as nodular fasciitis, and contributes to the range of appearances seen in other pathological conditions (El-Labban et al., 1996). Myofibroblast-like cells might sometimes be derived from vascular smooth muscle cells or from pericytes or by metaplasia. Some fibroblastic lesions have a component of myofibroblasts, but myofibroblasts do not apparently differentiate to smooth muscle cells. In wound healing, as epithelialization is completed, the myofibroblasts are presumed to disappear by apoptosis. Myofibroblasts are short, bi- or tripolar spindle-shaped or stellate cells, with crenellated or ovoid pale-staining nuclei each of which has a single distinct, dotted nucleolus (Feiner et al., 1976; Gabbiani et al., 1976). There is sparse cytoplasm with indistinct cell margins. In addition to synthesizing collagens and other stromal components including fibronectin and laminin, myofibroblasts have contractile elements showing α-actin and vimentin.

MATERIALS AND METHODS

Biological specimens were harvested from two patients one diagnosed with fibro-inflammatory epulis and one diagnosed peripheral giant cell granuloma.

Immunocytochemical detection for α-actin and vimentin was performed by LSAB method. Paraffin sections have been performed at 3 microns thickness. Paraffin was removed by 3 baths of xylol and rehydration by increasing alcohol concentrations, then rinsed in PBS for 20 minutes. Antigen retrieval was performed by proteinase K, sections were incubated in primary antibody at appropriate dilution in antibody dillution buffer overnight. After rinsing in PBS for 20 minutes, peroxidase blocking was performed by specific solution for 10 minutes at room temperature; rinsing sections for 20 minutes in PBS. The secondary biotinilated antibody, diluted in PBS was applied for 30 minutes at room temperature. Detection was performed by streptavidin alkaline peroxidare for 20 minutes at room temperature. Counterstaining was performed by Mayer haematoxylin. After that the sections were rinsed in PBS, dehydrated, cleared in xylene then mounted in glycerol, and examined in an Olympus BX40 with 8mpixels CCD camera and image acquisition software.

RESULTS

For the fibro-inflammatory epulis biological specimens, the immunolabelling with vimentin showed an important level in connective tissue cells in the deep chorion (fig.1), Positive vimentin cells have been observed also in the cells near an abundant chronic inflammatory reaction (fig.2). In some specimens, the positive vimentin cells have a dominant perivscular position.

In the same clinical case, on the same samplex, the immunolabelling with α-actin was positive for myofibroblasts in the deep chorion (fig. 3, 4), in the media of the mid-blood vessels (fig.5,6,7) around the inflammatory process.

For the peripheral giant cell granuloma samples, immunolabelling with vimentin showed the presence of

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the positive connective cells near an abundant chronic inflammation area (fig. 8). Positive cells for vimentin were found mainly in a perivascular position.

The same samples showed immunolabelling with α-actin in specific areas near the muscle wall of mid-vessels or near the inflammatory process.

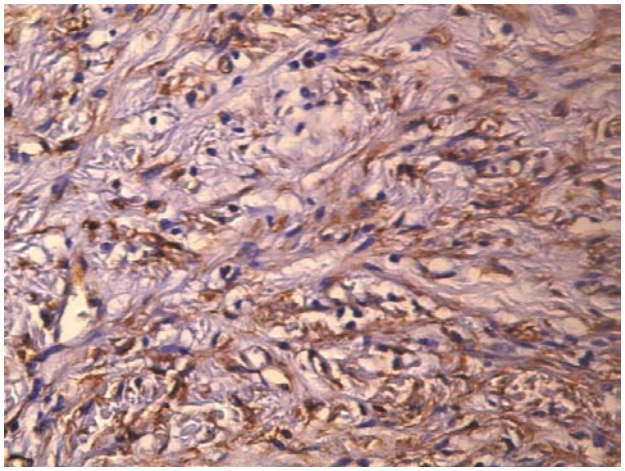

Fig. 1 Deep chorion, vimentin positive connective cells. Detail. (IHC_vimentin, x 40)

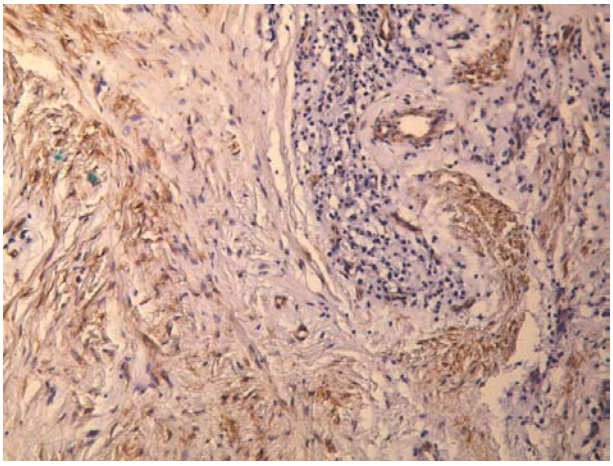

Fig. 2 Inflammatory process with vimentin positive cells between inflammatory cells and near them (IHC_vimentin, x 20)

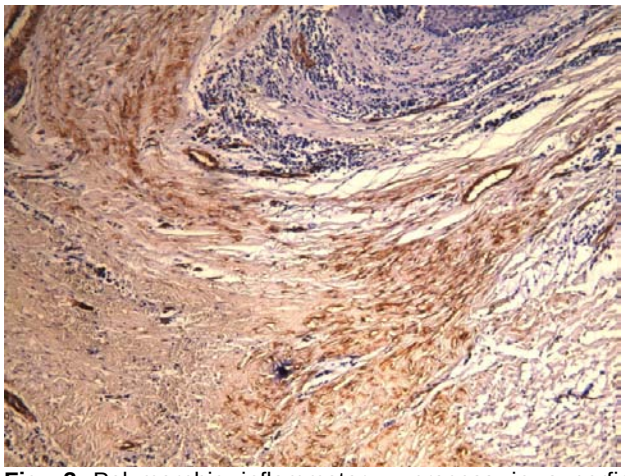

Fig. 3 Polymorphic inflammatory process in superficial chorion, while in the deep chorion we can observe fibroblasts disseminated between the collagen fibers (IHC_actin, x 10)

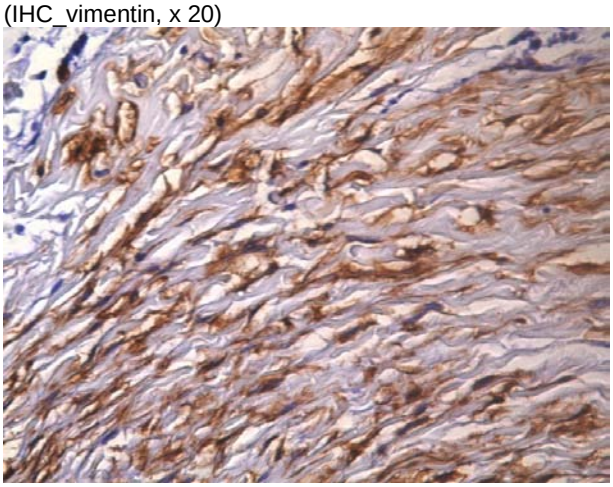

Fig. 4 Detail for the positive labelling with α-actin for myofibroblasts (IHC_actin, x 40)

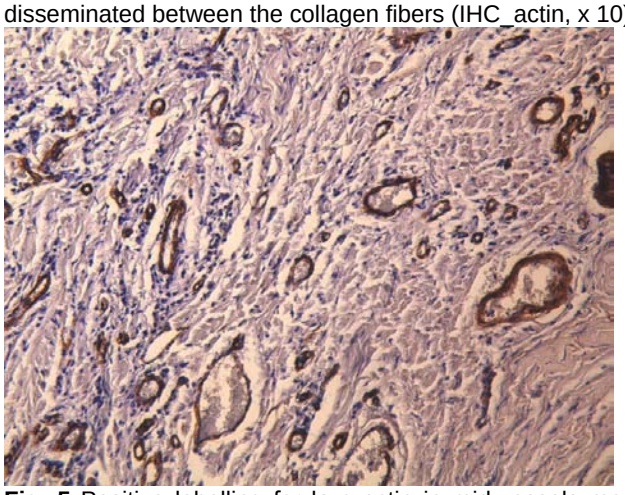

Fig. 5 Positive labelling for la α-actin in mid-vessels media (IHC actin, x 20)

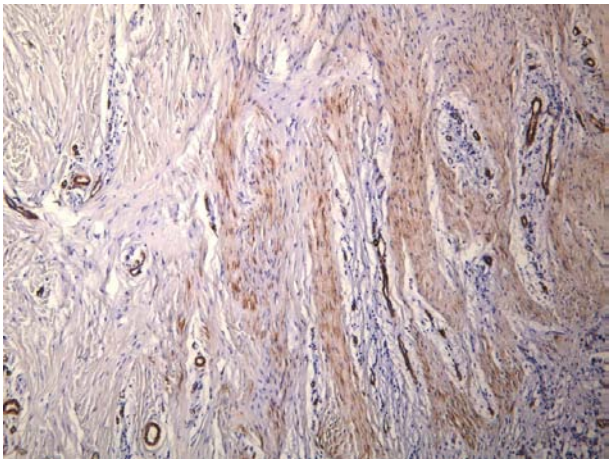

Fig. 6 Cells positive for α-actin, in the vessels walls and in disseminated myofibroblasts (IHC, actin, x 20)

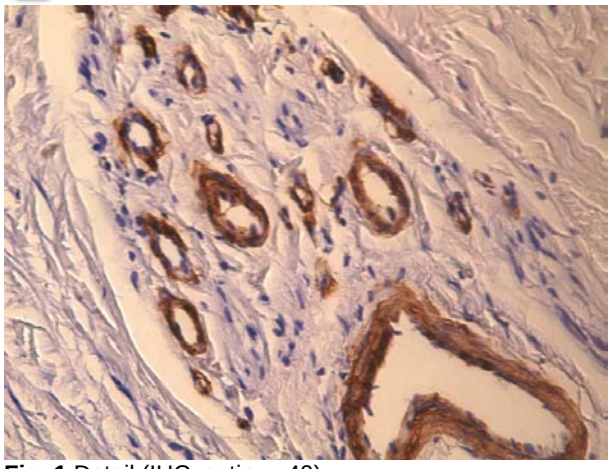

Fig. 1 Detail (IHC_actin, x 40)

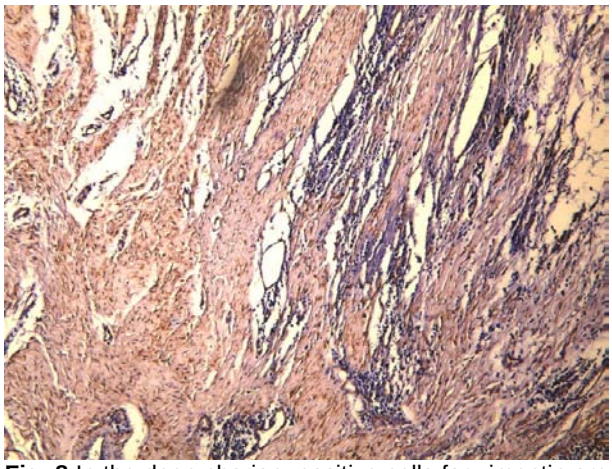

Fig. 8 In the deep chorion, positive cells for vimentin are located near an abundant chronic inflammatory reaction (IHC_vimentin, x 10)

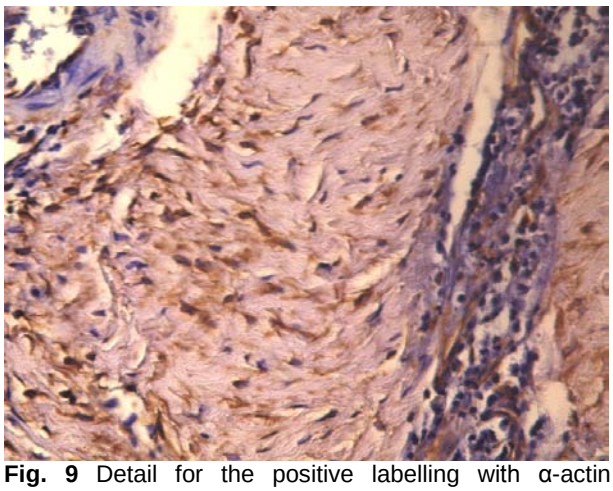

Fig. 9 Detail for the positive labelling with α-actin for myofibroblasts (IHC_vimentin, x 40)

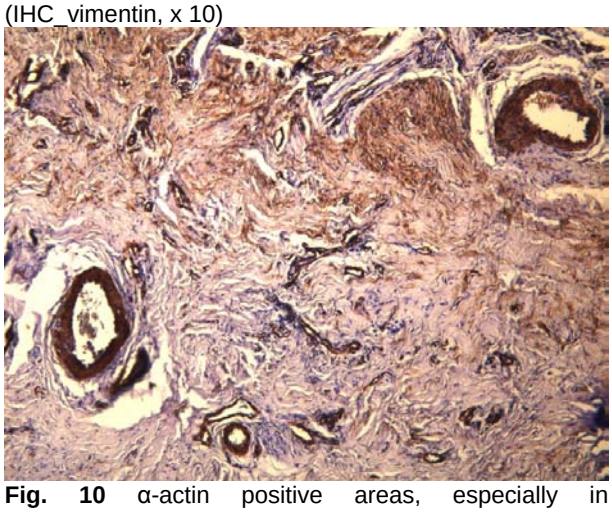

Fig. 10 α-actin positive areas, especially in myofibroblasts near the chronic inflammatory reaction and in mid vessels walls (IHC_actin, x 10)

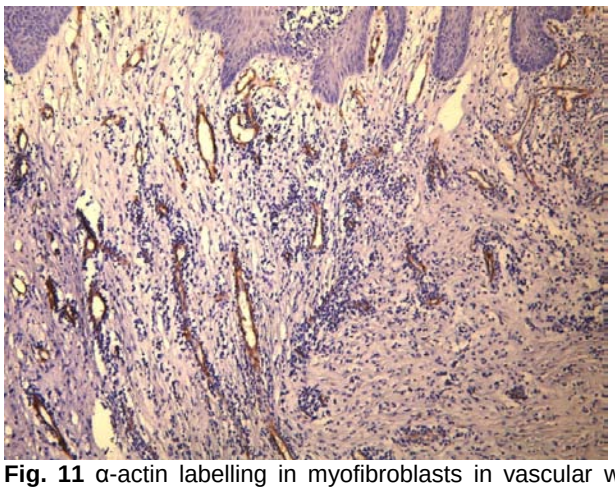

Fig. 11 α-actin labelling in myofibroblasts in vascular walls (IHC_actin x 10)

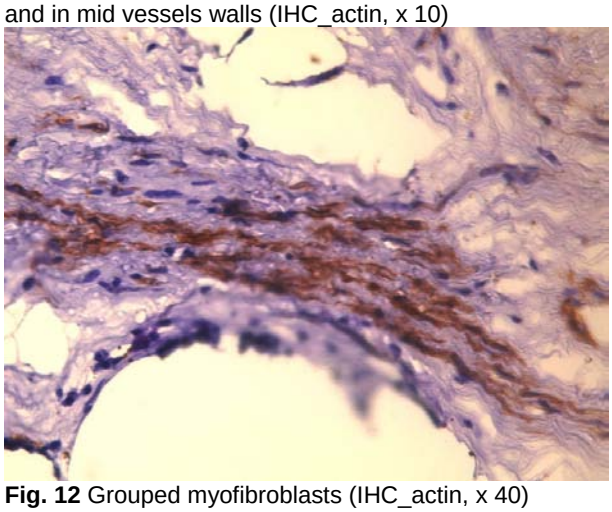

Fig. 12 Grouped myofibroblasts (IHC_actin, x 40)

DISCUSSION AND CONCLUSIONS

In the present study we have investigated the immunocytochemical labelling for vimentin and actin in connective tissue benign tumours: fibro-inflammatory epulis and peripheral giant cell granuloma samples. Myofibroblasts were present in the tumour samples, isolated or grouped, near and inside the inflammatory areas. In epulis lesions participate all elements in the connective tissue (vessels, cells, and fibres) while the epithelial compounds shows only a reactive area characteristic for benign tumours.

In the fibro-inflammatory epulis, labeling for vimentin was important for the connective cells in the deep chorion but positive vimentin cells were observed

also near chronic inflammatory reaction. Actin immunolabelling was positive for the myofibroblasts in the deep chorion, in the smooth muscle cells in the mid-vessels walls or in the myofibroblasts near the inflammatory process.

In peripheral giant cell granuloma, vimentin labelling was present near the inflammatory areas and perivascular while α-actin labelling was positive in myofibroblasts near the inflammatory process.

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