Stromal Myofibroblast Density in Dentigerous Cyst, Odontogenic Keratocysts and Ameloblastoma: An Immunohistochemical Study

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Abstract

Aims and objectives: This study aimed to analyze immunohistochemically the density of myofibroblast (MF) in dentigerous cyst (DC), odontogenic keratocyst (OKC) and ameloblastoma (AB).

Methods: Thirty formalin-fixed, paraffin-embedded specimens of DC, OKC and AB, ten each, were included in the present study. Immunohistochemical staining with anti-α-SMA primary antibody was performed. Area fraction of immunopositivity was measured by image analysis software followed by statistical analysis using Kruskal Wallis and post-hoc Dunn's tests.

Results: All cases of the three lesions were immunopositive for α-SMA. The median area fractions were 0.57, 8.72 and 17.87 for DC, OKC and AB respectively with a statistically non-significant difference between AB and OKC and statistically significant differences between AB and DC and between OKC and DC. A positive correlation was observed between the density of MF and the known biologic behavior of the lesions.

Conclusion: Over-expression of α-SMA found in AB and OKC might be used as a reliable marker for aggressiveness.

Keywords: ameloblastoma, dentigerous cyst, odontogenic keratocysts, myofibroblast.

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Introduction

Odontogenic cysts and tumors are those lesions that arise in the jaws from the odontogenic tissue remnants. They obviously vary in both size and severity although benign in nature [1].

Dentigerous cyst (DC) is a totally benign and non-aggressive cyst that enlarges by expansion and fluid accumulation. It is the most common cyst after the radicular cyst. It is always surrounding a crown of an impacted tooth arising from the reduced dental epithelium that surrounds the crown [2].

Odontogenic keratocyst (OKC) is an exceptional dilemmatic epithelial benign locally invasive odontogenic lesion. It passed through many classifications, once as a cyst then described as a tumor then classified as a cyst again. All this dilemma is returned to its peculiar microscopic picture, aggressive clinical behavior and abnormal genetic profile [3].

Ameloblastoma (AB) is the most common odontogenic tumor after odontoma. Despite its benign nature, it is locally invasive and shows a high tendency for recurrence after removal. It consists of odontogenic epithelium within a mature fibrous stroma [4].

Odontogenic lesions are composed of proliferating epithelium along with a complex tissue composed of both noncellular (matrix proteins) and cellular components (tumor-associated fibroblasts, endothelial and inflammatory cells) [5].

Oliveira et al. in 2004[6], displayed the importance of the supporting stroma and its interaction with the neoplastic epithelium, such as the stromal cells secretions of many factors to boost tumor growth and invasion.

A myofibroblast (MF) is an altered fibroblast that shows some smooth muscle features such as formation of contractile stress fibers and expression of α-smooth muscle actin (α-SMA). It is found physiologically in the connective tissue stroma of blood vessels, bone marrow, lymph nodes, alveolar septa and interstitial pericryptal cells [7].

A fibroblast converts to a MF by passing through two stages. In the first stage, a fibroblast gains contractile β and γ cytoplasmic actin bundles and focal adhesions so called proto-myofibroblast. Then in the second stage, the proto-myofibroblast gains α-SMA incorporated into its β-actin fibers to become a mature MF [8].

Material and Methods

I. Case Selection

The material of the present study consisted of thirty formalin-fixed, paraffin-embedded specimens of DC, OKC and AB, ten cases from each lesion. The specimens were collected from the archives of the General Pathology Departments, Faculties of

Medicine, Ain Shams University and Cairo University and Oral Pathology Department, Faculty of Dentistry, Ain Shams University.

II. Immunohistochemical Staining and Assessment

Immunostaining was performed on deparaffinized 4-*um* sections after antigen retrieval using microwave oven heating with citrate buffer retrieval solution (pH 4.8).

Anti-α-SMA monoclonal concentrated antibody (MC0004, Lab Vision corporation, USA) was used.

For immunostaining, the avidin-biotin-peroxidase complex method was used according to manufacturer instructions. In brief, after deparaffinization and inactivation of endogenous peroxidase activity and blocking of cross reactivity using blocking solution (hydrogen peroxide block), the sections were incubated with the primary antibody overnight in a humidity chamber at room temperature. Localization of the primary antibody was achieved by subsequent incubation of biotinylated anti-primary antibody with an avidin-biotin complex conjugated to horseradish peroxidase and diaminobenzidine. After incubation, the slides were washed twice with phosphate buffered saline. Counterstaining was performed using Mayer's hematoxylin. Negative control was formed by substituting the primary antibody with phosphate-buffered saline.

Immunohistochemical evaluation was carried out using the image analysis software (Image J, 41a NIH, USA) to measure the mean area fraction.

III. Statistical Analysis

Data were analyzed using Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA). Data were presented as median and interquartile range (IQR). Kruskal Wallis followed by post-hoc Dunn's was used for comparing quantitative non-parametric data of more than two different groups.

Results

All examined cases of DC, OKC and AB showed positive α-SMA immunoexpression. The reaction was confined to the nuclei and cytoplasm of MFs as well as the blood vessels.

DC showed a few α-SMA immunopositive MFs in the connective tissue wall of the cyst either directly beneath the epithelial lining or deep in the cyst wall (Fig. 1).

OKC showed more prominent α-SMA immunopositive MFs in the subepithelial zone and lower one or two thirds of the cystic wall (Fig. 2).

All examined cases of AB showed strong α-SMA immunoexpression. The distribution of MFs was diffuse in the connective tissue stroma of the neoplasm around the neoplastic epithelial follicles and toward the invasive front of the lesion (Fig. 3).
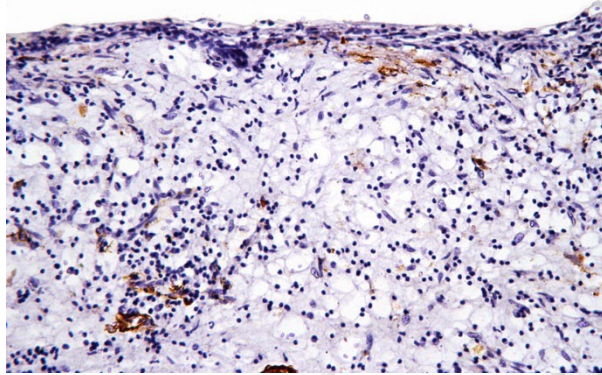

Fig.1: A photomicrograph of DC showing positive nuclear and cytoplasmic α-SMA immunostaining apparent in few MFs and wall of blood vessels (original magnification 40X).
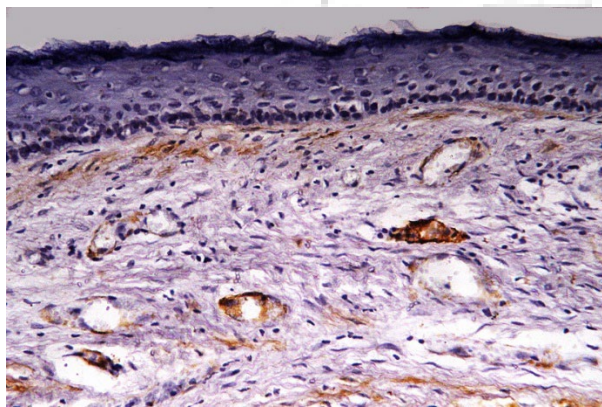

Fig.2: A photomicrograph of OKC showing positive nuclear and cytoplasmic α-SMA immunostaining in many MFs as well as the wall of the blood vessels (original magnification 40X).
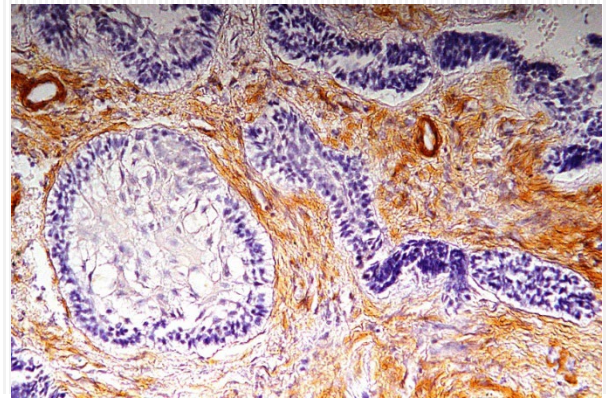

Fig.3: A photomicrograph of AB with positive nuclear and cytoplasmic α-SMA immunostaining in MFs and blood vessels around the neoplastic epithelial follicles (original magnification 40X).

There was a statistically significant difference between median area fraction of α-SMA expression in the three lesions (P-value < 0.001). Pair-wise comparisons revealed that AB showed a statistically non-significant higher value of α-SMA median area fraction (17.85) than OKC (8.72) (P=0.054) and a statistically significant higher value than DC (0.57) (P<0.001). DC showed the lowest median α-SMA expression with a statistically significant difference from OKC (P=0.004) (table 1).

Table (1): Kruskal Wallis test followed by post-hoc Dunn's for comparison of α-SMA in different studied groups (Median-IQR).

	DC	OKC	AB	P value
Median	0.57	8.72	17.85	<0.001*
IQR	0.35-0.68	5.38-10.20	11.54-27.60	

P value <0.05 is considered significant

Discussion

Most of odontogenic lesions, which include cysts and neoplasms are benign in nature, although they differ greatly in their behavior and severity. DC, which is the most common developmental odontogenic cyst, is totally benign with no tendency for invasion or recurrence, unlike OKC, which shows a clinical aggressive behavior and high tendency for recurrence. This aggressive behavior is also found in AB which is considered one of the most common odontogenic neoplasms in the oral cavity [5,9].

Former researches supposed that the appearance of MFs in the wall of the odontogenic cysts is a normal response to the distension caused by the enlargement of the cyst [10].

Nowadays, recent researchers found that the stroma of the OKC and odontogenic tumors is not only for support but also plays a great role in the neoplastic behavior. Presence of MFs in the stroma is a neoplastic phenomenon caused by secretion of transforming growth factor β (TGF-β) and platelet derived growth factor (PDGF) by neoplastic epithelial cells [11].

In this study, α-SMA immunoexpression was positive in all of the examined cases. In DC, a few α-SMA positive cells were distributed in the connective tissue wall of the cyst either directly under the epithelium of the cyst or deeply seated in the cyst wall. Lombardi and Morgan, 1995 [10] referred the presence of a few MFs directly under the cystic epithelium and deep in the cyst wall to the strain exerted by positive hydrostatic pressure from cystic fluid on the cyst wall providing the environment for the conversion of stromal fibroblast to MF.

In OKC, the α-SMA positive cells were distributed predominantly in the subepithelial zone and lower third or two thirds of the cystic wall. In accordance with current results, Priya et al., 2014 [12] and Goel et al., 2019 [13] observed a similar distribution pattern of α-SMA immunoexpression in OKC. This distribution could clearly explain the role of MFs in progression and infiltration of the lesion rather than part of the host defense mechanism against invasion as was thought previously.

In AB, the distribution of α-SMA positive cells were diffuse but more concentrated around the neoplastic epithelium and near the bone trabeculae in the infiltrative border of the lesion. This was in accordance with Goel et al., 2019 [13] who elucidated that by the role of MFs in the degradation of the adjacent matrix, facilitating tumor island growth, expansion and invasion of the surrounding structures.

In addition to the stromal expression of α-SMA in the present lesions, vascular

expression in the walls of the blood vessels was also noticed. Prabakar et al., 2016 [14] explained this vascular reaction by expression of α-SMA in endothelial cells, pericytes, smooth muscle cells as well as the MFs surrounding the blood vessels. In this study, these vascular reactions were considered a positive control. Quantitatively, a statistically non-significant higher α-SMA immunoexpression was noticed in AB compared with OKC with a statistically significant higher α-SMA immunoexpression in AB compared with DC and in OKC compared with DC. This was in agreement with Syamala et al., 2016 [9] and in contrary to the study of Ali et al., 2018 [5] who found a statistically significant higher difference in α-SMA immunoexpression in AB than in OKC. On the other hand, Eid et al., 2018 [15] found a slightly higher α-SMA expression in OKC than AB with a statistically non-significant difference. They attributed this to the similar proliferative potential of both AB and OKC. Lewis et al., 2004 [16] elucidated that MFs may promote tumor progression and invasion by different ways. They upregulate the expression of serine and matrix metalloproteinases, which degrade and remodel extracellular matrix, facilitating cell invasion and migration. MFs also secrete interstitial matrix, as well as numerous soluble mediators of inflammation and growth factors, including hepatocyte growth factor/scatter factor which is identified as a potent mitogen for hepatocytes, but also dissociates and induces motility of epithelial cells in carcinomas.

It could be concluded from this study that over-expression of α-SMA found in AB and OKC might be used as a reliable marker for invasiveness and aggressiveness.

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