ORIGINAL ARTICLE

Evaluation of stromal myofibroblasts expression in keratocystic odontogenic tumor and orthokeratinized odontogenic cysts: A comparative study

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ABSTRACT

Objective: Keratocystic odontogenic tumor (KCOT) has an aggressive clinical course and a high tendency of recurrence, while orthokeratinized odontogenic cyst (OOC) has different characteristics and does not show aggressive behaviour. Even the treatment of these two lesions varies considerably. A large number of epithelial molecules have been studied in order to differentiate odontogenic keratocyst (OKC) from OOC, but stromal factors have not been adequately studied. Recently, tumor stroma has evolved as a particular field of interest. In the present study, we aim to evaluate and compare the expression of stromal myofibroblasts (MFs) in these entities and correlate it to its aggressive behavior. The term 'keratocystic odontogenic tumor' has been introduced by WHO in 2005 for odontogenic keratocyst keeping in mind its aggressive behavior, however still many pathologists and clinicians use the term OKC synonymously. **Materials and Methods:** A total of 10 cases of KCOT and 10 cases of OOC were stained for alpha-smooth muscle actin (αSMA) for demonstration of stromal MFs. MF frequency was assessed as the number of αSMA-positive stromal cells in 10 high power fields, presented as the mean number of positive cells per field. **Results:** Counts showed that the mean number of positive cells in KCOT (20.6 ± 2.05) was significantly higher than that seen in OOC (10.4 ± 1.06) ($P < 0.05$). **Conclusion:** The different behaviors of these lesions are compatible with the finding of the present study. The increased number of stromal MFs in KCOT in comparison to OOC correlates with its aggressive behavior and increased tendency towards recurrence.

Key words: Keratocystic odontogenic tumor, orthokeratinized odontogenic cyst, stromal myofibroblasts, αSMA

INTRODUCTION

Keratocystic odontogenic tumor (KCOT), a developmental abnormality from derivatives of the dental lamina, has an aggressive clinical behavior and a propensity towards recurrence. It arises sporadically or in association with the nevoid basal cell carcinoma syndrome (NBCCS).[1]

It was first described by Philipsen in 1956,[2] and is now designated by the World Health Organization (WHO) as KCOT and is defined as "a benign uni- or multicystic, intraosseous tumor of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and potential for aggressive, infiltrative behavior."[3] WHO "recommends the term keratocystic odontogenic tumor as it better reflects its neoplastic nature."[3] Orthokeratinized odontogenic cyst (OOC), has originally been described by Philipsen[2] as a possible type of OKC; but is now considered as a distinct entity.[4]

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The typical histology of KCOT includes lesions whose epithelial lining is uniformly thin, ranging from 8 to 10 cell layers. The basal layer exhibits a characteristic palisaded pattern with uniform nuclei and the luminal epithelial cells are often parakeratinized. Orthokeratinized foci, however, can also be found. Occasionally, budding of the basal cell layer into surrounding connective tissue and the formation of microcysts can be seen. The fibrous cyst wall is relatively thin and usually lacks inflammatory cell infiltrate. While OOC demonstrates a orthokeratinized surface and a

prominent granular layer lying immediately below the flat surface.[5]

In contrast to KCOTs, the OOC has no tendency to recur and it is not associated with the NBCCS.[6] The higher recurrence rate, aggressive behavior, and neoplastic potential of KCOT suggest the importance of distinguishing KCOT and OOC. Recent investigations have demonstrated significant differences in the histological features and clinical behavior of these entities.[1] A wide range of epithelial-associated factors are implicated in the relative aggressive biological behavior of the odontogenic epithelium, but only a few studies have investigated nonepithelial factors that could contribute to the variable biological behavior of different types of odontogenic cysts and tumors.[7] It is now well-accepted that the coordinated activity of epithelial cells with their stroma is fundamental in controlling growth and differentiation in normal and pathological situations.[8] The role of tumor stroma in tumor progression is an important area of current research and has become a potential target for therapeutic intervention.[9] Presence of stromal MFs, an important component of tumor stroma has been linked to the biological behavior of both benign and malignant tumors.[10,11] Vered *et al*., in a immunohistochemical (IHC) study assessed the frequency of MF in different odontogenic cysts and tumors and correlated it to their aggressive biological behavior.[7]

The aim of the present study was to quantitatively assess and compare the number of stromal MFs in KCOT and OOC.

MATERIALS AND METHODS

Study cases

Formalin-fixed, paraffin-embedded blocks of 10 cases each of KCOT and OOC were retrieved from the archives of the Department of Oral Pathology. Diagnoses were established on the hematoxylin and eosin (H and E) stained-slides [Figures 1 and 2]. Included cases were devoid of considerable inflammatory infiltrate in cystic walls.

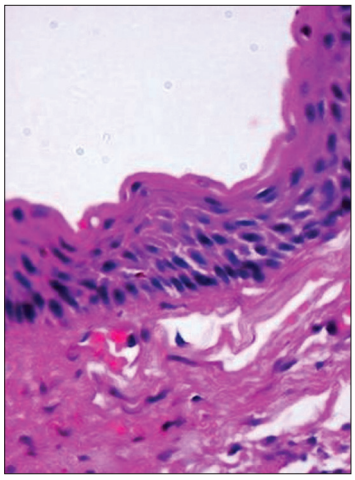

Figure 1: Lining of KCOT showing palisaded basal layer of tall columnar cells with reversal of nuclear polarity. The surface exhibits parakeratinization. Subepithelial connective tissue shows radially arranged collagen fibers (H&E stain, ×200)

Staining procedure

Sections, 3 μm thick, were mounted on silane coated slides. After dewaxing in xylene, sections were dehydrated in ethanol, rinsed in distilled water, placed in 3% H_2O_2 for 10 min, and rinsed in distilled water for 15 min. For antigen retrieval procedure, slides were placed in citrate buffer solution, pH = 6, in a microwave at 92°C for 10 min. After cooling at room temperature for 20 min, slides were exposed to primary alpha-smooth muscle actin (αSMA; marker for MFs) mouse anti-human antibody (Biogenex Ltd.) of dilution 1:100, for 60 min at room temperature. Slides were rinsed in phosphate buffer solution for 10 min. For antibody detection, universal immune peroxidase polymer anti-mouse rabbit HistofineR (Multi) kit (Nichirei, Tokyo, Japan) was used. Sections were rinsed in phosphate buffered saline (PBS) for 10 min, reacted with 3-Amino-9-Ethylcarbazole (AEC) substrate-chromagen kit, rinsed in PBS for 2 min, counterstained in Mayer's hematoxylin, and mounted with nonaqueous mounting agent.

Histomorphometric evaluation of αSMA in stained sections

Representative fields were randomly selected in each immunohistochemically stained section.

Counts were performed with a BH-2 Olympus microscope ×10 ocular, ×40 objective, and a counting grid containing 100 squares that determined the perimeter of the chosen field. Ten fields were chosen for each section. The grid was placed immediately beneath the cystic epithelial lining. Each αSMA-positive cell, excluding those surrounding blood vessels, was counted and the total number of positive cells for all 10 examined fields per case was calculated. This allowed calculation of the mean number of αSMA-positive cells per field.

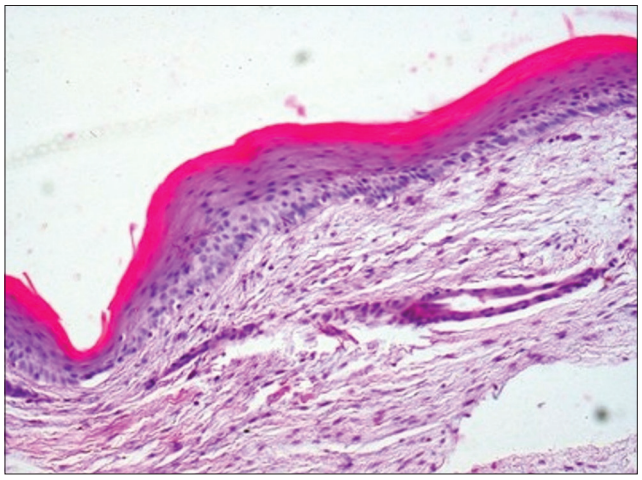

Figure 2: Orthokeratinized odontogenic cyst: Stratified squamous epithelial lining with surface thick layer of orthokeratin (H&E stain, ×100)

Statistical analysis

Difference in the mean number of αSMA-positive cells per field between KCOT and OOC were analyzed using independent Student's *t*-test. Statistical significance was at $P < 0.05$. The Statistical Package for the Social Sciences (SPSS 14) software was used for computations.

RESULTS

The mean number of αSMA-positive cells per field in all examined cases of KCOT and OOC is shown in Table 1. Spindle cells showing fine αSMA-positivity were located beneath and parallel to the basement membrane of the odontogenic epithelium of these cystic lesions [Figures 3 and 4]. Additional small aggregates and short, delicate bundles of similar cells were found within the fibrous wall. αSMA positive cells within blood vessel walls served as positive control for the specificity of the stain.

The value of αSMA positive cells in KCOT (20.6 ± 2.05) was significantly higher than that seen in OOC (10.4 ± 1.06) ($P < 0.05$) [Table 2].

DISCUSSION

The purpose of this study was to evaluate and compare the stromal MFs in KCOT and OOC. We found that the mean number of stromal MFs per high power field was considerably higher in the KCOTs.

KCOT is an aggressive cystic lesion that has a tendency to recur if not adequately removed. The recurrence rates have been documented with variable results from 3 to 60%. The recurrence rates of KCOT and OOC were studied and the results showed that KCOT recurred in at least 42.6%, compared with only 2.2% for the OOC. These data suggested the importance of distinguishing KCOT and OOC.[6]

Several attempts have been made to clinically, histologically, and biologically distinguish KCOT from OOC. A large series of the study revealed that OOC was more often associated with an impacted tooth than KCOT.[6] However, there were no significant differences between OOC and KCOT when age, race, sex, presenting symptoms, and the clinical impression were compared. Histologically, KCOT has a thin wall unless there is inflammation.[12]

The basal layer of the epithelium is well-defined and is composed of either columnar or cuboidal cells arranged in a palisaded pattern. The luminal surface, often corrugated, is typically covered with parakeratin. The OOC has a thin, uniform epithelial lining with a luminal surface of orthokeratin and well-developed granular layer.[4] Basal cells of the OOC

Table 1: Mean number of alpha-smooth muscle actin positive cells per case

Case	KCOT	OOC
1	20.5	9
2	22	11
3	21.2	10.7
4	19.8	9.4
5	23	10.3
6	22.7	8.9
7	18	11.3
8	19.3	10
9	22.5	12
10	17	11.4

KCOT: Keratocystic odontogenic tumor, OOC: Orthokeratinized odontogenic cyst

Table 2: Mean±standard deviation for KCOT and OOC

Lesion	Mean±standard deviation
KCOT	20.6±2.05
OOC	10.4±1.06

KCOT: Keratocystic odontogenic tumor, OOC: Orthokeratinized odontogenic cyst

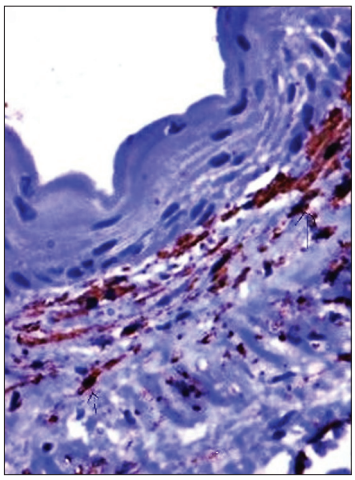

Figure 3: KCOT: Subepithelial connective tissue shows alpha-smooth muscle positive cells radially arranged parallel to each other (IHC stain, ×200)

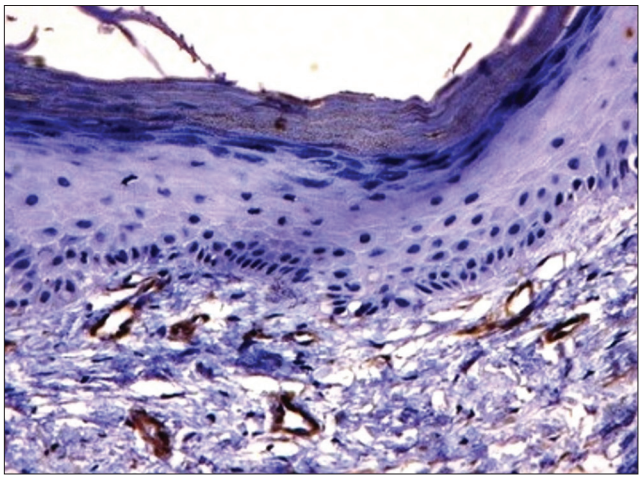

Figure 4: αSMA positive cells present only in the wall of blood vessels, while subepithelial connective tissue shows very few scattered cells. (IHC stain ×200)

are much less developed than those in the KCOT. They tend to be cuboidal or squamous and show little tendency to polarize or palisade. It was suggested that care must be taken to distinguish between keratin metaplasia in otherwise non-keratinized odontogenic cysts and the OOC.[4]

The proliferative activity of the lining epithelium of KCOT has been the subject of various investigations aiming at the expression of p53, proliferating cell nuclear antigen (PCNA), and Ki67. Such studies concluded that p53, PCNA, and Ki67 are more strongly expressed in KCOTs than in other types of odontogenic cysts.[13] Thosaporn *et al*., indicated that the proliferation index of IPO-38 was useful + in predicting the different biological behavior of the odontogenic lesions. Moreover, the KCOT should be regarded as benign tumor, while the OOC as a nonaggressive cystic lesion.[14]

Rangiani and Motahhary in their study showed that there is a significant difference between the two cysts regarding bcl-2 proapoptotic protein expression that is compatible with their different clinical behaviors.[1] Recently, the IHC profiles of cytokeratins 10, 13, and 14 and extracellular matrix proteins, fibronectin, types I and III collagen and tenascin, indicated that the OOC presented a well-formed cystic enveloping, whereas the KCOT profile was compatible with a more aggressive biological behavior.[15] However, till date there are only few studies evaluating the role of the stromal cells in the aggressiveness of these cystic lesions.

Stromal involvement in tumor progression was examined by extracellular matrix components in syndrome and non-syndrome OKC[16] and showed differences in expression of tenascin, fibronectin, and collagen IV, explaining, in part, the different degrees of aggressiveness of biological behavior of these entities. In an additional study, collagen in the walls of OKCs was analyzed histochemically by staining the sections with picrosirius red and examining them with polarizing microscopy.[17] It was found that the staining of the collagen fibers in the KCOTs was similar to that reported in the odontogenic neoplasms,[18] suggesting that the stroma of KCOT could be regarded not just a structural support of the cyst wall, but as the one playing a part in the neoplastic behavior of the cyst as well.

In general stromal reaction to epithelial neoplasm is marked by the appearance of MFs.[19] Approximately 30 years ago, MF were shown to be present in the stroma of various invasive and metastatic malignant tumors characterized by hard consistency and retraction (e.g., ductal mammary carcinoma associated with skin or nipple retraction and metastatic carcinoma to lymph nodes fixed to surrounding tissue and overlying skin). At that time it was assumed that this phenomenon was part of the host reaction to prevent invasion of malignant cells, since MF were numerous, particularly at the invasive front. However, over the past 10 years, there has been an abundance of evidence that the presence of MF at the invasion front is not part of the host defence mechanism against tumor invasion, but actually promotes it.[7]

These cells have also been reported in a number of pathological states involving the oral tissues: Nodular fascitis, giant cell fibroma, malignant fibrous histiocytoma, gingival hyperplasia, central and peripheral giant cell granulomas, and adult and infantile fibromatosis.[20] Stromal MFs are known to remodel the extracellular matrix and helps in its degradation by secretion of matrix metalloproteinases thereby promoting the invasive growth of epithelial lesions.[8,21]

The presence of MF in odontogenic lesions has not been thoroughly investigated. Vered *et al*., evaluated quantitavely the expression of MFs in different odontogenic cysts and tumors, and the results showed that the mean number of MF, in well-recognized aggressive odontogenic lesions (ameloblastoma and OKC) was high and did not differ significantly from that in SCC. In contrast, known nonaggressive lesions (unicystic ameloblastoma, ameloblastic fibro-odontoma, OOC, and dentigerous cyst) showed significantly lower results compared to ameloblastoma and OKC. They suggested a positive link, that is when more MFs are present in the stroma, a more aggressive behavior of the odontogenic cyst/tumor can be anticipated.[7]

Our findings showed that the mean number of αSMA positive cells in the connective tissue wall of KCOT was significantly higher than that in OOC and stated that the two cysts not only differ in the epithelial characteristics, but also in the stromal wall component. This study is also an evidence for the distinction between the two cysts as separate lesions, and categorizing KCOT as a cystic tumor; while the OOC as a nonaggressive cystic lesion.

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