Morphometric evaluation of keratocystic odontogenic tumor before and after marsupialization

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Abstract: The aim of the present study was the morphometric evaluation of the epithelial lining and fibrous capsule in histological specimens of keratocystic odontogenic tumors (KOTs) before and after marsupialization. Histological sections from six KOTs that had undergone marsupialization followed by enucleation were photographed. The thickness and features of the capsule and of the epithelial lining of the tumor were evaluated upon marsupialization and upon subsequent enucleation using Axion Vision software. The histological specimens taken upon marsupialization presented an epithelial lining that is typical of KOTs. After marsupialization, the enucleated specimens had a modified epithelial lining and a fibrous capsule that both presented a greater median thickness (p = 0.0277 and p = 0.0212, respectively), morphological changes, and significant enlargement. These modifications can facilitate full surgical treatment and may well be related to a low KOT recurrence rate.

Descriptors: Surgery, Oral; Odontogenic Tumors; Histology.

Introduction

A keratocystic odontogenic tumor (KOT) is a benign neoplasm of the jaws that originates either from a dental lamina or from the primordial odontogenic epithelium.[1] KOTs were first described by Phillipsen,[2] who named them "odontogenic keratocysts". The aggressive clinical behavior and high recurrence rate of KOTs suggest a true neoplastic potential, which led the World Health Organization in 2005 to classify them as benign tumors containing an odontogenic epithelium with a mature and fibrous stroma and no odontogenic ectomesenchyme.[1]

KOTs are more commonly found in the mandible than in the maxilla,[3,4] especially in the posterior maxillary region.[3-6] This tumor affects individuals of both genders,[3] with a slight predilection for males,[6,7] and shows a higher incidence in the third decade of life, with an almost equal distribution in the other decades.[3] Histopathologically, KOTs are characterized by a connective tissue capsule lined with parakeratinized stratified squamous epithelium of 5–8 cell layers. The basal layer consists of columnar epithelial cells with hyperchromatic nuclei arranged in palisades. The epithelial-connective tissue junction is flat without epithelial projections.[2]

The treatment of KOTs includes decompression;[8,9] marginal resection;[5] *en bloc* resection;[10] and adjuvant therapy, such as cryotherapy,[11] pe-

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Table 1 - Patient data and clinical features of KOTs (n = 6).

Patient	Gender	Age (years)		GGS[1]	Site	M/E[2] (months)		Follow-up duration (months)		Recurrence
		Patient	Mean			Patient	Mean	Patient	Mean	
1	M	33	21.3 ± 9.6	No	LMd[3]	14	11.6 ± 4.8	46	35.1 ± 6.7	No
2	F	20		Yes	RMx[4]	17		26		No
3	M	20		No	LMd	10		33		No
4	F	14		Yes	LMd	14		36		No
5	M	9		Yes	RMd[5]	3		38		No
6	M	32		No	LMd	12		32		No

[1] GGS, Gorlin-Goltz syndrome; [2] M/E, time between marsupialization and enucleation; [3] LMd, left mandible; [4] RMx, right maxilla; and [5] RMd, right mandible.

ripheral ostectomy,[7,12,13] and Carnoy's solution.[3,13,14] The adjuvant therapies have been applied to reduce recurrences.[3] The KOT recurrence rate varies from 5% to 62%.[8] Several authors report that marsupialization conducted to reduce the dimensions of extensive KOTs promotes a second, less complex procedure with low morbidity.[4,8,9,15,16] In addition, prior studies have reported that after marsupialization, the fibrous capsule becomes thicker and less friable, facilitating the surgical procedure of enucleation and reducing the recurrence rate.[4,9,16] The histological evaluation of the epithelial lining also showed metaplasia.[17] However, to date, no study has morphometrically evaluated the epithelial lining and fibrous capsule of KOTs at different times during their treatment with marsupialization. Therefore, the aim of the present study was to conduct a morphometric analysis of the epithelial lining and fibrous capsule in histological sections of KOTs before and after marsupialization.

Methodology

Six patients with KOTs who underwent marsupialization (incisional biopsy) followed by enucleation were included in this study. After marsupialization, the reduction in the size of their KOTs was examined quarterly through clinical and radiographic examinations. The time between marsupialization and enucleation in each case is shown in Table 1. The enucleation was performed when a dimensional reduction of the tumor could not be radiographically identified within a 3-month period. After enucleation, the surgical sites of all the patients were treated using peripheral ostectomy. Subsequently, Carnoy's solution was applied (consisting of 60% ethanol, 30% chloroform, and 10% glacial acetic acid) for 5 minutes. This study was approved by the Research Ethics Committee of the *Universidade Federal de Minas Gerais* - UFMG (COEP/UFMG -15/08), and all of the participants signed an informed consent form.

The histological sections of selected KOT cases were retrieved from the files of the Oral Pathology Service when the diagnosis proved to be in accordance with the findings of Barnes *et al.*[1] All samples were fixed in 10% buffered formalin and processed for hematoxylin-eosin staining (Sigma-Aldrich, Steinheim, Germany). The thickness of the epithelial lining and fibrous capsule at the time of marsupialization and after enucleation was measured using Axion Vision imagery analysis software, version 4.7.1 (Imaging Systems, Carl Zeiss, Oberkochen, Germany). The images of the specimens were viewed under a standard 25 microscope (Carl Zeiss, Oberkochen, Germany) with a final magnification of 100× and photographed using a Canon Power Shot A-640 digital camera (Canon Inc., Tokyo, Japan). The photographs were transferred to the software, and the thickness measurements were conducted and recorded by a single examiner. The analyses of the images of each specimen were performed separately for the epithelial linings and the fibrous capsules, maintaining the surface of the epithelial lining parallel to the border of the picture frame so that

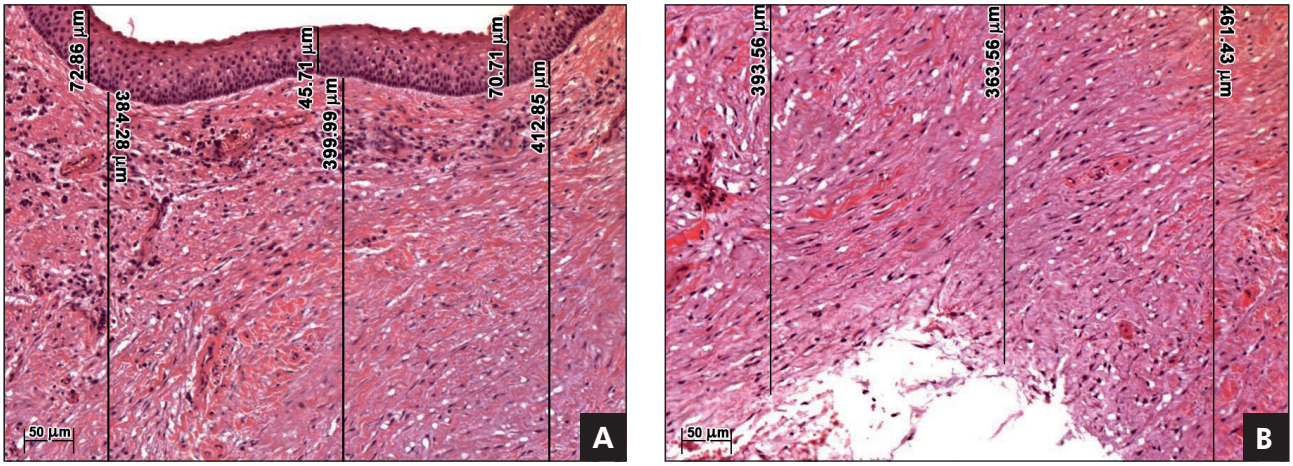

Figure 1 - Keratocystic odontogenic tumor specimen before marsupialization. **A:** The epithelial lining is represented by parakeratinized stratified squamous epithelium with few layers and a basal palisade layer with cubic or columnar cells and hyperchromatic nuclei. The epithelial-connective tissue junction is flat. **B:** Sequential measurements of the fibrous capsule shown in Figure 1A. The fibrous capsule consists of dense, sparsely cellularized, and slightly vascularized connective tissue. The three measurements (upper, middle, and lower) of the epithelial lining and fibrous capsule are represented by black lines (hematoxylin-eosin stain, 100× original magnification).

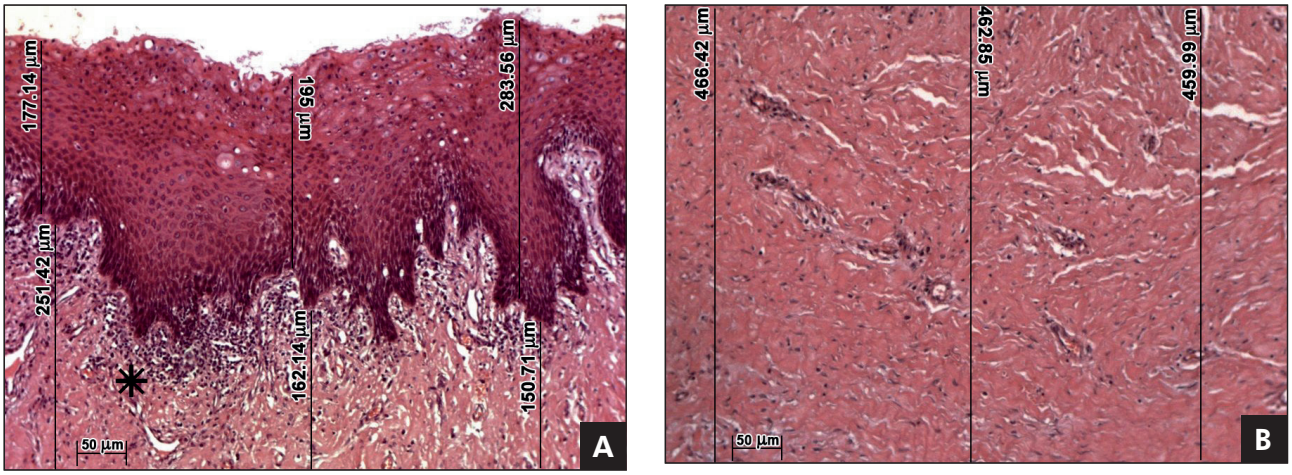

Figure 2 - Keratocystic ontogenic tumor specimen after enucleation. **A:** The epithelial lining is represented by nonkeratinized, hyperplastic stratified squamous epithelium. The three measurements (upper, middle, and lower) of the epithelial lining and fibrous capsule are represented by black lines (hematoxylin-eosin stain, 100× original magnification). **B:** Sequential measurements of the fibrous capsule shown in Figure 2A. The fibrous capsule consists of dense, sparsely cellularized, and slightly vascularized connective tissue with chronic inflammation (asterisk). The three measurements (upper, middle, and lower) are represented by black lines (hematoxylin-eosin stain, 100× original magnification).

the measurements could be taken perpendicularly to the basal layer (Figures 1A and 1B).

When the total thickness of the fibrous capsule could not be determined from a single image, sequential images were taken (Figures 1A, 1B, 2A, and 2B). The images were taken until all of the specimens had been included. Three measurements (upper, middle, and lower) were performed on each image (Figures 1A, 1B, 2A, and 2B.) The mean values were recorded and expressed in µm. The BioEstat (BioEstat software version 4.0, Belém, Brazil) statistical package was used to analyze the data, applying the Wilcoxon test and Student's t-test to the data from the epithelium and the fibrous capsule,

Table 2 - Epithelial lining thickness of KOTs in the specimens obtained from marsupialization (MA) and enucleation (EN; n = 6).

Specimen	MA (μm)	EN (μm)	Thickness increase (%)
1	80.79	199.76	147.25
2	263.17	601.66	128.62
3	96.96	297.34	206.66
4	86.57	228.36	163.78
5	26.20	113.70	333.96
6	93.18	190.81	104.77
Mean	107.81 ± 2.08	271.93 ± 1.74	180.84 ± 82.51
Median	89.87*	214.06*	155.51

*Statistically significant difference ($p = 0.0277$, Wilcoxon test).

respectively. The probability value established for statistical significance was $p < 0.05$. The morphology of the epithelial lining and fibrous capsule was also observed and recorded both before and after the marsupialization.

Results

The data regarding the patients and the clinical features of their KOTs are described in Table 1.

In all cases, it was clinically obvious upon enucleation that the appearance of the tumor had changed from the time of marsupialization, with a considerable thickening of the tumor's fibrous capsule. This change facilitated the surgical procedure.

The histologically prepared specimens of the lesions were lined by parakeratinized stratified squamous epithelium containing layers of only approximately 5–6 cells before the marsupialization procedures. The basal layer consisted of cuboidal or columnar epithelial cells with hyperchromatic nuclei arranged in palisades. The epithelial-connective tissue junction appeared flat with no epithelial projections (Figure 1A). The specimens obtained after marsupialization showed stratified squamous epithelium, which was predominantly nonkeratinized and hyperplastic, at times forming projections into the capsule. In addition, lymphocytic exocytosis and spongiosis could also be observed (Figure 2A). Four specimens presented focal areas of classic epithelial morphology associated with KOTs. The thickness measurements of the epithelium during marsupialization ranged from 26.2 to 263.17 μm (median of 89.87 μm), whereas the thickness in the KOT specimens after enucleation ranged from 113.7 to 601.66 μm (median of 214.06 μm). This thickness difference was statistically significant ($p = 0.0277$, Wilcoxon test). Comparing the measurements taken during marsupialization and during enucleation, the epithelium presented a mean increase in thickness of 180% (Table 2).

The fibrous capsule in the specimens from the marsupialization procedure consisted of dense, cellularized, slightly vascularized connective tissue, at times with focal areas of inflammation. Microcysts could not be observed on the incisional or enucleation specimens. In the specimens from the enucleation procedure, the fibrous capsule consisted of dense and slightly cellularized connective tissue, with a variable amount of mononuclear inflammatory infiltrate (Figures 2A and 2B). The fibrous capsules from the incisional biopsy (marsupialization, 1395.01 μm ± 2.05) were not as thick as the capsules obtained from the final enucleation (3562.15 ± 1.71, $p = 0.0212$, Student's t-test; Table 3). Therefore, the marsupialization procedure increased the thickness of the fibrous capsule by 294%.

Discussion

Previous studies have suggested that the lining of a keratocyst is only 5 or 6 cells thick and tears easily during enucleation, in turn, causing a high recurrence rate. However, after decompression or marsupialization, the lining appears to become thicker and easier to enucleate; however, histologically, the lining appears to change and resembles that of the normal oral mucosa.[4,8,9] Nevertheless, to date, only

Table 3 - Fibrous capsule thickness of KOTs in specimens obtained from marsupialization (MA) and enucleation (EN, n = 6).

Specimen	MA (μm)	EN (μm)	Thickness increase (%)
1	1044.84	1478.92	41.54
2	3163.85	5446.77	72.15
3	1036.15	1884.64	81.88
4	526.09	3794.56	621.27
5	2063.86	3532.72	71.17
6	535.32	5235.32	877.97
Mean	1395.01 ± 2.05*	3562.15 ± 1.71*	294.33 ± 362.14
Median	1040.49	3663.64	77.01

*Statistically significant difference ($p = 0.0212$, Student's t-test).

descriptive studies have been performed to evaluate changes in the epithelial lining and fibrous capsule of KOTs at different times during marsupialization treatment. In the present study, a morphometric evaluation demonstrated that the epithelium and fibrous capsule in the specimens from enucleation were statistically significantly thicker than in the specimens from the incisional biopsy. This finding scientifically confirms the clinical observations.[4,8,16]

The histopathology of the incisional biopsies presented the classic features of KOTs. After marsupialization, the features of the epithelium and fibrous capsule were modified. Similar changes have also been observed in other studies.[3,4,9,16] Pogrel[9] reported that the covered tumor was altered with marsupialization and acquired the characteristics of normal oral mucosa in both routine histopathological and immunohistochemical analyses. The inflammatory infiltrate identified by surgery can render the appearance of KOTs similar to that of normal oral mucosa. In KOTs having some degree of inflammation, Stoelinga[3] observed a loss of epithelial keratinization that resembled that seen in radicular, inflamed dentigerous, and paradental cysts. De Paula *et al.*[18] observed a predominantly nonkeratinized epithelial lining and an increased proliferative activity of inflamed compared with noninflamed KOTs. August *et al.*[19] also noted a moderate hyperplasia and inflammation in many tumors that had been subjected to decompression. These authors also demonstrated that the altered expression of cytokeratin 10 is a marker of epithelial changes that occur in large KOTs submitted to marsupialization. Marker *et al.*[16] found changes in satellite cysts when the cysts occurred in the fibrous capsule of KOTs subjected to marsupialization. This phenomenon was not observed in the tumors sampled in the present study. August *et al.*[19] observed that the satellite cysts, upon cystectomy, continued to express both cytokeratin 10 and KOT features, despite the differentiation of the main portion of the lesion.

One unexpected finding in the current study was the difference in the initial epithelial thickness of patient 5 compared with the thickness found for the other patients (Table 2). This patient had the greatest variation in thickness before and after marsupialization (333.96%, Table 2) over the short term (3 months, Table 1). Future studies with a larger sample of patients are warranted to examine the relationships of age at the diagnosis of KOT, physiological status, and individual genetic characteristics with the behavior of these lesions after marsupialization.

In the present study, the observations at the time of enucleation demonstrated that the appearance of the tumor changes after marsupialization. A thickening of the capsule covering the tumor could be observed, which facilitated the detachment of the entire lesion from the bone walls and its surgical removal. Marker *et al.*[16] and Pogrel[9] observed the same alteration in a group of patients with KOTs treated by marsupialization. During the inspection of the surgical cavity in all cases, smooth bone walls but no remaining tumor could be observed. Despite

apparent tumor removal, adjuvant treatment of surgical cavities with peripheral ostectomy and Carnoy's solution was established as protocol.[13]

Alternatives have been proposed for the treatment of KOTs, and currently, the selection of more conservative procedures is advocated. The present study applied marsupialization under local anesthesia.[6] This technique involves the cutting of an opening in the wall of the lesion and the suturing of the tumor covering to the oral mucosa.[8] A polyethylene drain is routinely used to maintain communication between the inner tumor and the oral cavity. This drain is anchored by tying it with steel wire to the teeth adjacent to the surgical opening. Other studies have described the use of an acrylic shutter as an alternative, especially in edentulous patients, as well as nasopharyngeal probes to maintain marsupialization devices.[4,9,16]

Pogrel and Jordan[8] distinguishes between decompression and marsupialization. However, the biological mechanism in both cases is the same, i.e., the reduction of the pressure within the lesion and establishment of contact between the surface of the tumor and the oral mucosa. The use or omission of a device to maintain the patency of the surgical opening depends on the anatomic features of the region.

Reducing the size of the KOT by marsupialization facilitates its removal,[4,9,16] as observed in the current study. However, the option of marsupialization generally requires the patient to undergo a second surgical procedure to enucleate the remaining lesion and to thus endure a longer treatment.[4,5,16,19] A mean of 11.6 months elapsed between the incisional biopsy and the enucleation in the six evaluated patients. No problems could be identified during this period. Therefore, it can be concluded that a longer treatment time is not a disadvantage of marsupialization.

The maintenance period after marsupialization varied among the patients in this study from 3 to 17 months (mean of 11.6 months). During this time, the thickening of the fibrous capsule blocked the inner portions of the lesion and prevented its continued remission. Therefore, the total regression of the lesion after marsupialization could not be identified, as reported by Pogrel and Jordan.[8] Marker *et al.*,[16] who also based their findings on radiographic evaluations, delayed approximately 10 months before performing enucleation and determined that longer delays promote more changes in the lining of tumor, which may be related to a decreased aggressiveness of the lesion. August *et al.*[19] suggest a treatment period of at least 9 months prior to enucleation to produce epithelial differentiation. Brondum and Jenses,[4] using radiographic evaluation, found that the period from marsupialization to enucleation ranged from 1 to 14 months.

The literature reports that the recurrence rate of KOTs is between 0%[5] and 62%.[20] The capsule of thin connective tissue and the plane junction between the epithelial and connective tissues cause the tumor wall to be friable. Moreover, cyst satellites can contribute to the recurrence of KOTs.[5,10] Several authors recommend a long period of postoperative follow-up.[5,6,15] Woolgar *et al.*[21] warn that the development of a new tumor unrelated to the previous one in an adjacent region of the jaw can sometimes be interpreted as a recurrence. Furthermore, the KOTs that affect patients with Gorlin-Goltz syndrome commonly behave more aggressively,[7] most likely due to the higher rate of epithelial proliferation that occurs in these patients.[22] In the present study, no recurrences could be observed among the evaluated patients, including the syndromic patients.

Conclusion

In the present study, the epithelial lining and the fibrous capsule of KOTs were morphologically altered and significantly enlarged after marsupialization. These modifications facilitate full surgical treatment and may well be related to a low KOT recurrence rate.

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