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Original Article

Clinicopathological study of radicular cysts with actinomycosis

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Background/purpose: Actinomycosis is sometimes associated with a radicular cyst (RC). This retrospective study evaluated the clinical and histopathological features of 6 RCs with actinomycosis (AM/RCs).
Materials and methods: The demographic data and clinicopathological features of 6 AM/RCs were collected and analyzed.
Results: The 6 AM/RCs were taken from 2 male and 4 female patients, and 3 were found in the maxilla and the other 3 in the mandible. The involved teeth included maxillary or mandibular incisors (2 cases) and maxillary or mandibular first and second molars (4 cases). The most common symptom was pain (5 cases) and the more frequent signs were sinus tract and pus discharge (4 cases). Microscopically, the actinomycotic colony presented as a mass with filamentous bacteria arranging in a sun-ray pattern at the periphery. The mean actinomycotic colony number was 7.7 ± 6.6 colonies per slide. Due to the severe inflammation in all 6 AM/RCs, the stratified squamous epithelial lining was completely abolished in 2 cases and partially destroyed in 4 cases with the residual epithelial lining varying from approximately 10%–50%.
Conclusion: Our results indicate that pain is the most common symptom and sinus tract and pus discharge are the two frequent signs of our 6 AM/RCs. The stratified squamous epithelial lining was either completely abolished (2 cases) or partially destroyed (4 cases) in 6 AM/RCs. Thus, if the endodontically-treated tooth shows a recurrent sinus tract and poor response to repeated

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conventional root canal treatments, periradicular actinomycotic infection should be highly suspected.

Introduction

Radicular cyst (RC) is the most common odontogenic cyst and the second most frequent periapical lesion next to periapical granuloma.[1,2] RC is defined as an epithelium-lined cystic lesion at the periapical area of a nonvital tooth.[3] The source of the epithelium is usually the epithelial rest of Malassez that is stimulated by inflammation to proliferate and further form a RC. Our previous study of the epithelial linings of 377 RCs and found that 367 (97.3%) of the 377 RCs are lined by nonkeratinized stratified squamous epithelium.[4]

There are two types of RC: periapical true cyst and pocket cyst.[5] A periapical true cyst is self-sustaining as the lesion is no longer dependent on the presence or absence of root canal infection. Therefore, the true cysts, particularly the large ones, are less likely to be resolved by nonsurgical root canal treatment. Periapical pocket cysts, particularly the smaller ones, may heal after nonsurgical root canal therapy.[5] However, if the periapical pocket cyst is infected by actinomycotic bacteria, it often does not respond well to nonsurgical endodontic treatment and needs a further periapical surgery and a short-term systemic antibiotic administration to cure the periapical infection.[6–10]

Understanding the causes of endodontic treatment failure is of paramount importance for the proper management of this condition. Teeth with post-treatment apical periodontitis can be managed by either nonsurgical endodontic retreatment or periradicular surgery, both of which can result in restoring the health of the periradicular tissues and maintaining the tooth function in the oral cavity.[5] The major etiology of post-treatment apical periodontitis is intraradicular infection persisting in the complex apical root canal system, but in some cases a secondary intraradicular infection may arise from coronal leakage or missed root canals.[5,10] In addition, an extraradicular infection, especially the periapical actinomycosis, may be the major extraradicular cause of endodontic treatment failure.[5–10] Thus, it is interesting to know the clinical and pathological features of RCs when they had concurrent actinomycotic infection.

Actinomycosis is a rare, chronic, and slowly progressive granulomatous disease caused by filamentous Gram-positive anaerobic bacteria from the Actinomycetaceae family (genus Actinomyces).[11] Orocervicofacial actinomycosis is the most common form of the disease and comprises about 50% of all reported cases.[11] The actinomycotic bacteria can also cause periapical infection through the infected root canal of a tooth, which is also called as periapical actinomycosis.6-8 Pain, inflammation, tissue destruction, swelling, abscess, and sinus tract formation are common symptoms and signs of orocervicofacial actinomycosis and periapical actinomycosis.[6–11] Therefore, this study tried to evaluate and report the demographic data, the clinical features (including involved teeth, endodontic treatment history, clinical diagnosis, patients' symptoms and signs, and radiographic characters), and histopathological features (including the presence of actinomycotic colonies, the number of actinomycotic colonies per slide, the epithelial lining, and acute and chronic inflammatory cell infiltrates in the fibrous cystic wall) of 6 RCs with actinomycosis (AM/RCs). In addition, we assessed whether there were intimate correlations between the clinical features and histopathological features of these 6 AM/RCs.

Materials and methods

The AM/RC cases included in the study had to fulfill all the following criteria: 1) the histopathological diagnosis of a RC with available demographic and clinical records and tissue slides or blocks, 2) a periapical or a panoramic radiograph showing a periapical radiolucency of an involved tooth or several involved teeth, 3) a description of seeing a periapical cystic lesion in the operation record, 4) a microscopic finding of the presence of actinomycotic colonies mainly in the cystic lumen. All the AM/RC cases were retrieved from the archives of a private pathological center, Taipei, Taiwan from August 2020 to February 2021. During this period, 922 biopsy tissue specimens including 445 tissue specimens of periapical lesions (197 cases of periapical granuloma, 239 cases of radicular cyst, 8 cases of periapical scar, and one case of periapical abscess) were sent to the pathological center for histopathological diagnosis. The age and gender of patients, the involved tooth/teeth, clinical diagnosis, patients' symptoms and signs, and radiographic features of the AM/RCs were reviewed and recorded from the pathological diagnosis requisition sheets.

All surgical specimens were obtained from curettage or enucleation of the cystic lesions. The removed tissue specimens were immediately placed into 10% neutral formalin and fixed for at least 24 h. They were then dehydrated in graded alcohol, and embedded in paraffin. The tissue blocks were cut in serial sections of 5 μm, and stained with hematoxylin and eosin (H&E). Two oral pathologists independently performed the review of the diagnosis and analysis of the histopathological features based on microscopic examination of the H&E-stained tissue sections. The microscopic criteria for diagnosis of a RC included the presence of a cystic cavity surrounded partially or completely by the lining epithelium and fibrous connective tissue wall. If the lining epithelium was not observed in the tissue section but the cystic cavity and inflamed fibrous connective tissue wall were easy to be identified and the finding of a cystic lesion during the operation was reported by the local dentist

performing the surgery, then it could still be diagnosed as a RC. Microscopic findings suggestive or characteristic of other known pathologic entities were excluded from the current study. A double-headed light microscope was used to reach a consensus in cases where inconsistent pathological diagnosis and findings were encountered.

Re-evaluation of the slides was focused on the presence of actinomycotic colonies in the cystic lumen, the number of actinomycotic colonies per slide, the type of epithelial lining (usually the stratified squamous epithelium), the percentage of residual epithelial lining (parts of the epithelial lining were destroyed due to severe acute or chronic inflammation or both in the underlying fibrous connective tissue), and the presence of foamy histiocytes, cholesterol clefts, and hemosiderin-laden macrophages in the fibrous cystic wall. We also assessed the type of inflammation (acute or chronic) and the degree of chronic inflammation (classified as mild, moderate, and severe depending on the amount of chronic inflammatory cell infiltrate) in the fibrous cystic wall. A diagnosis of mild, moderate and severe chronic inflammation was made if the extent of inflammatory cell infiltration was less than 25%, more than 25% but less than 50%, and more than 50% of the tissue sections, respectively.

Results

A total of 6 AM/RCs were identified from 239 cases of RC and included in this study. These 6 AM/RCs were taken from 6 patients (2 men and 4 women, mean age 52 ± 17 years, range 32–78 years). The male to female ratio was 1:2 (Table 1). Of the 6 AM/RCs, 3 were found in the maxilla and the other 3 in the mandible. The most frequently involved teeth were maxillary or mandibular incisors (2 cases) and maxillary or mandibular first and second molars (4 cases). Five cases of AM/RCs each involved one tooth and the remaining one involved two teeth (Table 1). In this study, five patients had a long and complicated endodontic history that showed failure after repeated nonsurgical endodontic treatments. The clinical diagnoses for the 6 AM/RCs were RC in 3 cases, periapical granuloma in one (case 4), chronic periodontitis or breast cancer metastasis in one (case 3), and chronic osteomyelitis in one (case 6) (Table 1). The clinicians did not suspect periradicular actinomycosis in any one of these 6 AM/RCs. For the symptoms of 6 AM/RCs, mild, moderate or severe pain was noted in 5 cases and one was asymptomatic. Regarding the signs of 6 AM/RCs, swelling, sinus tract, and pus discharge were found in 3 cases, sinus tract and pus discharge but without swelling in one case, and mobility of involved tooth in 2 cases (Table 1). Radiographically, 4 AM/RCs were presented as a well-demarcated periapical radiolucent lesion and the remaining two as an ill-defined radiolucent lesion: one was suspected to have a breast cancer metastasis because the patient had a past medical history of breast cancer (case 3), and the other was found to have chronic osteomyelitis (case 6). The size of the lesion ranged from approximately 5 mm–20 mm in greatest dimension (mean, 11.7 ± 5.2 mm).

Histopathological features of 6 AM/RCs are shown in Table 2 and Figs. 1 and 2. Criteria for the diagnosis of actinomycotic colonies included filamentous bacteria aggregated to form a mass with variations in the color between the center and periphery of the colony in H&E-stained tissue sections. At the high-power view, the peripheral area of an actinomycotic colony exhibited deep blue filamentous bacteria arranging in a characteristic sun-ray pattern (Fig. 1). In addition, periodic acid Schiff (PAS) and Gram stains were sometimes used to confirm the diagnosis of actinomycosis, because actinomycotic colonies

Table 1 Demographic data and clinical features of 6 radicular cysts (RCs) with actinomycosis.

RC with actinomycosis	Patient age (year)	Patient gender	Involved tooth or teeth	Failure after repeated endodontic treatments	Clinical diagnosis	Patient symptoms	Clinical signs	Character and size of periapical radiolucent lesion (mm)
Case 1	38	Female	Teeth 21 and 22	+	RC	Mild pain	Swelling, sinus tract, pus discharge	Well-defined 15 × 12
Case 2	32	Female	Tooth 26	+	RC	Mild pain	Sinus tract, pus discharge	Well-defined 20 × 20
Case 3	61	Female	Tooth 46	_	Chronic periodontitis or breast cancer metastasis	Moderate pain	Tooth mobility	Ill-defined 10 × 8
Case 4	57	Female	Tooth 37	+	Periapical granuloma	None	Swelling, sinus tract, pus discharge	Well-defined 10 × 10
Case 5	78	Male	Tooth 41	+	RC	Severe pain	Tooth mobility	Well-defined 5 × 5
Case 6	45	Male	Tooth 27	+	Chronic osteomyelitis	Mild pain	Swelling, sinus tract, pus discharge	Ill-defined 10 × 10

Table 2 Histopathological features of 6 radicular cysts (RCs) with actinomycosis.

RC with actinomycosis	Actinomycotic colonies per slide	Residual epithelial lining	Fibrous cystic wall				
			Acute inflammatory cell infiltrate	Lymphoplasma cell infiltrate	Foamy histiocyte	Cholesterol cleft	Hemosiderin-laden macrophage
Case 1	6	None	+	severe	+	+	+
Case 2	1	None	+	severe	+	−	−
Case 3	20	30%	+	severe	+	−	−
Case 4	9	10%	+	severe	+	+	−
Case 5	6	30%	+	severe	+	−	−
Case 6	4	50%	+	severe	+	−	−

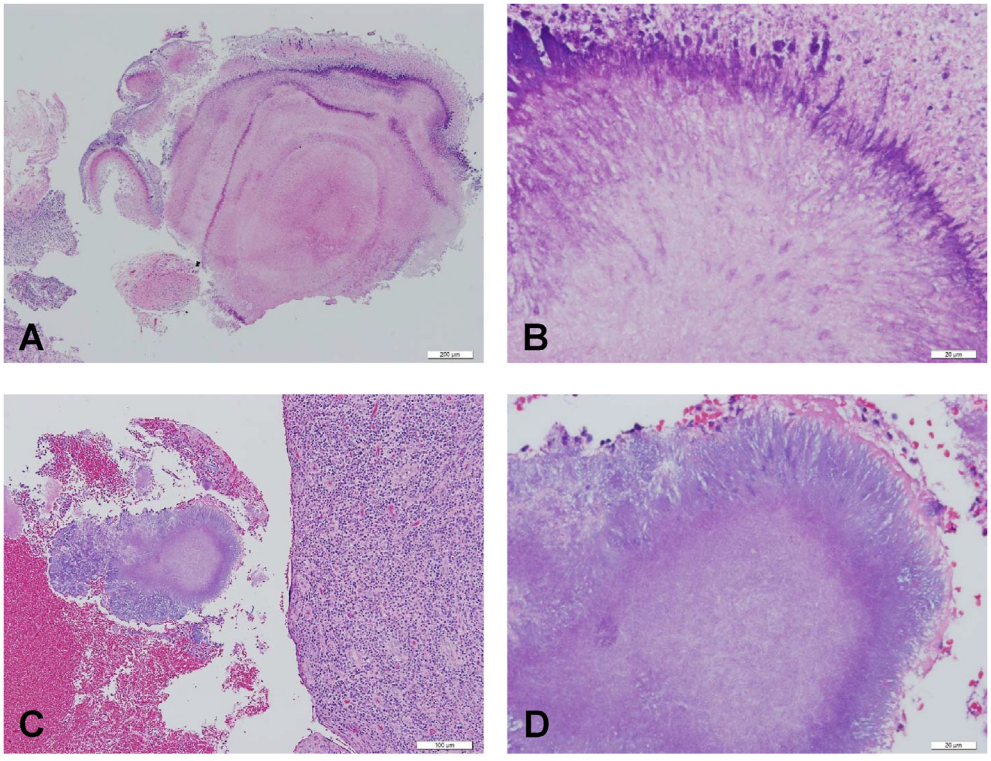

Figure 1 Histopathological features of actinomycotic colonies. (A) Low-power microphotograph showing several actinomycotic colonies in the lumen of a radicular cyst. The actinomycotic colonies revealed variations in the color between the center and periphery of each colony. (B) High-power microphotograph of the peripheral area of an actinomycotic colony exhibiting deep blue filamentous bacteria arranging in a characteristic sun-ray pattern. (C) Low-power microphotograph demonstrating an actinomycotic colony surrounded by aggregates of red blood cells in the lumen of a radicular cyst (left half) and the fibrous cystic wall of a radicular cyst with a severe lymphoplasma cell infiltrate but without an epithelial lining (right half). (D) High-power microphotograph of an actinomycotic colony showing the homogenous and slightly basophilic central part and the blue filamentous bacteria arranging in a specific sun-ray pattern at the peripheral part. (Hematoxylin and eosin stain; original magnification; A, 4×; B, 40×; C, 10×; D, 40×).

were positive for both PAS and Gram stains (data not shown). The actinomycotic colonies per slide varied from 1 to 20 colonies with a mean of 7.7 ± 6.6 colonies per slide (Table 2). Due to the presence of both severe acute and chronic inflammatory cell infiltrates (Fig. 2A, B, and C) in the subepithelial area of fibrous cystic wall in all 6 AM/RCs, the cystic stratified squamous epithelial lining was completely abolished in 2 cases (Fig. 2A) and partially destroyed in 4 cases with the residual epithelial lining varying from approximately 10%—50% (Table 2 and Fig. 2C). Foamy histiocytes (Fig. 2D), cholesterol clefts (Fig. 2E), and hemosiderin-laden macrophages (Fig. 2F) were found in the fibrous cystic wall of 6, 2, and 1 AM/RC, respectively (Table 2).

Discussion

In this study, 5 of the 6 patients had a long and complicated endodontic history showing treatment failure after

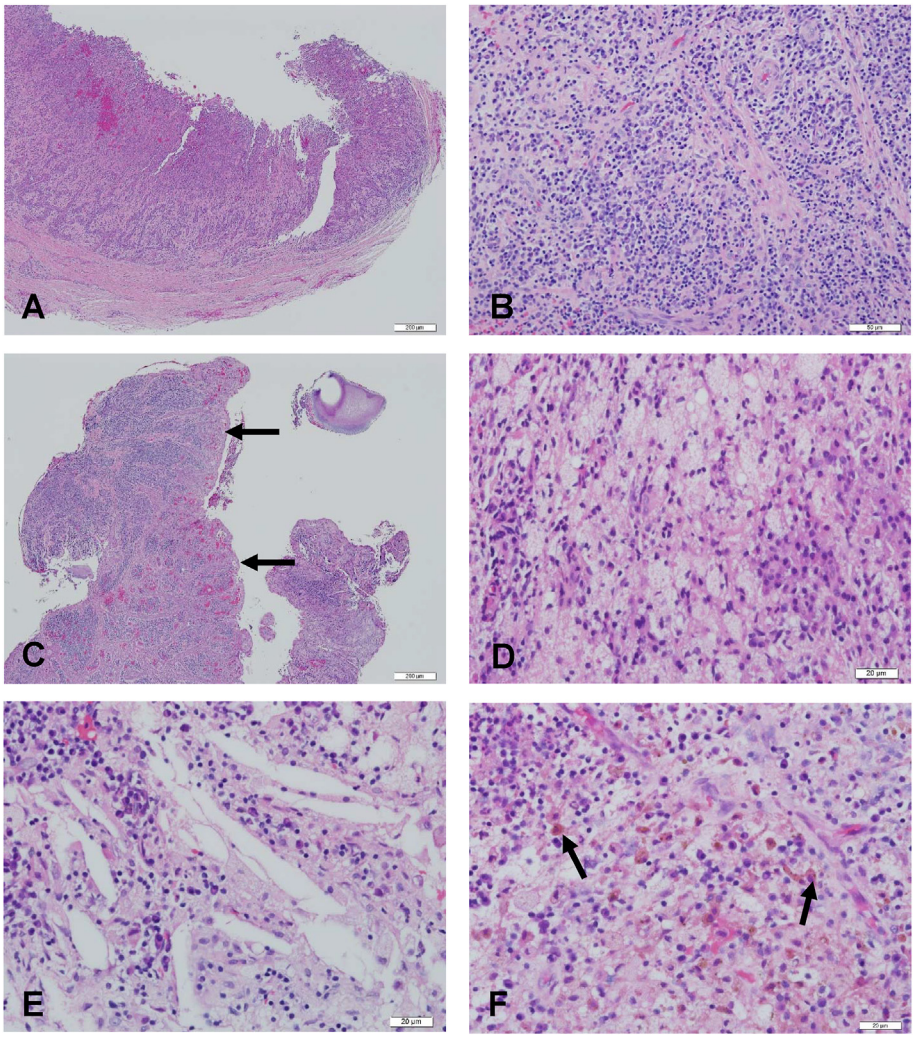

Figure 2 Histopathological features of radicular cysts with actinomycosis. (A) Low-power microphotograph exhibiting a well-encapsulated fibrous cystic wall of a radicular cyst. The outer one quarter of the cystic wall was composed of dense collagenous fibrous tissue with a very mild lymphoplasma cell infiltrate but the inner three quarters of the cystic wall was infiltrated by a sheet of lymphoplasma cells with foci of hemorrhage. No lining epithelium was found on the inner surface of the radicular cyst. (B) High-power microphotograph showing a severe lymphoplasma cell infiltrate in the fibrous cystic wall of a radicular cyst. (C) Low-power microphotograph showing a severely-inflamed radicular cyst with a stratified squamous epithelial lining (arrows) and an actinomycotic colony in the lumen of the radicular cyst (near right upper corner). (D, E and F) Aggregates of foamy histiocytes (D), cholesterol clefts (E), and scattered hemosiderin-laden macrophages (arrows) were discovered in the severely-inflamed fibrous cystic wall of a radicular cyst. (Hematoxylin and eosin stain; original magnification; A, 4×; B, 20×; C, 4×; D, E, and F, 40×).

repeated nonsurgical root canal therapies. This means that AM/RCs did not respond well to conventional nonsurgical endodontic treatments and further need a periapical surgery accompanied by a short-term antibiotic coverage to cure the lesion. Moreover, the AM/RCs were often combined with sinus tract formation and pus discharge (4 of the 6 AM/RCs), suggesting that infection with actinomycotic bacteria may cause severe inflammation, alveolar bone and cortical plate destruction, and finally formation of a sinus tract for pus discharge. Similar results were also reported by Happonen who studied 16 surgically-treated cases of periapical actinomycosis and found fistulation and abscess formation in 4 of the 6 RCs, 4 of the 8 periapical granulomas, and 2 of the 2 periapical abscesses.[7] The results of the above-mentioned two studies including ours indicate that periapical actinomycosis is more common than previously believed and these infections cannot be controlled by conventional nonsurgical endodontic treatments but can usually be cured by ordinary periapical surgery accompanied by a short-term (7—10 days) antibiotic coverage.[6—10] Moreover, actinomycosis can be observed in not only RCs but also periapical granulomas and periapical abscesses.[7]

For the symptoms of our 6 AM/RCs, three had mild pain, one moderate pain, one severe pain, and one none of symptom. The three AM/RCs with mild pain and one without pain symptom all had sinus tracts and pus discharge, suggesting that intermittent pus discharges from the sinus tract can relieve the pressure caused by severe inflammation, edema, and repeated production of pus and in turn reduce the painful sensation. The tooth with

moderate pain (case 3) had tooth mobility and a clinical diagnosis of chronic periodontitis because the periradicular radiolucency extended coronally to near the cementoenamel junction. Moreover, the periradicular radiolucency was ill-demarcated and suspected as a metastatic lesion of breast cancer due to the patient's past medical history of breast cancer. The tooth with severe pain (case 5) also had tooth mobility, this tooth was probably had chronic periradicular actinomycosis with acute exacerbation. The acute inflammation resulted in edema of the periodontal ligament and in turn led to severe pain and tooth mobility.

For the clinical diagnosis of our 6 AM/RCs, three were RC, one periapical granuloma, one chronic periodontitis, and the last one chronic osteomyelitis. However, the clinicians did not suspect periradicular actinomycosis in any one of these cases, suggesting that periradicular actinomycosis is still not included in the list of regular differential diagnosis of periapical lesions by the majority of practicing dentists including the endodontic specialists. For the last one with chronic osteomyelitis (case 6), the progression of chronic osteomyelitis led to gradual destruction of the alveolar bone around the involved tooth 27 and finally resulted in an ill-define radiolucent lesion.

The most characteristic histopathological feature of the 6 AM/RCs was the generalized destruction of the epithelial lining. The residual epithelial lining was usually less than 50% of the total epithelial lining with two cases showing none of the epithelial lining (Table 2). In this study, all 6 AM/RCs exhibited both severe acute and chronic inflammations, thus the lining epithelial cells were probably destroyed by the proteolytic enzymes released from the degenerating polymorphonuclear leukocytes in the cystic wall or lumen. The universal presence of foamy histiocytes in all 6 AM/RCs also indicates the persistent destruction of epithelial cells or inflammatory cells. The released cell debris including the lipid and cholesterol materials from the lytic cell membranes were subsequently phagocytosed by the histiocytes, leading to the formation of many foamy histicytes in the fibrous cystic wall. Regarding the two AM/RCs without epithelial lining, the fibrous cystic wall was characteristic of a RC with the gradual transition from the collagenous fibrous wall without inflammation into the inflammatory zone of the cystic wall (Fig. 2A). In addition, the clinicians found a cystic cavity during the surgical enucleation of the lesion. Therefore, it was not difficult to make a diagnosis of RC, even if no epithelial lining was found during the observation of the tissue section by microscopy.

Intraradicular infection persisting in the complex apical root canal system is the major cause of persistent apical periodontitis.[5] Other causes of persistent apical periodontitis that dental clinicians should bear in mind include extraradicular actinomycosis, periapical true cysts, foreign—body reaction to extruded root canal filling materials and endogenous cholesterol crystals, and scar tissue healing.[5] However, periradicular actinomycosis is still one important reason for failure of nonsurgical endodontic treatment.[5—10] Clinically, if the tooth shows a recurrent sinus tract and poor response to conventional nonsurgical endodontic treatments combined with antibiotic control, periradicular actinomycotic infection should be highly suspected.[6] Therefore, for AM/RCs, the proper treatment includes surgical curettage and a short course of antibiotic therapy. Healing was uneventful in all AM/RC cases after the above-mentioned proper treatments.[6]

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

References

1. Ricucci D, Mannocci F, Ford TR. A study of periapical lesions correlating the presence of a radiopaque lamina with histological findings. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:389—94.
2. Ramachandran Nair PN, Pajarola G, Schroeder HE. Types and incidence of human periapical lesions obtained with extracted teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996;81:93—102.
3. Neville BW, Damm DD, Allen CM, Chi AC. Pulpal and periapical disease. In: Neville BW, Damm DD, Allen CM, Chi AC, eds. *Oral and maxillofacial pathology*, 4th ed. St. Louis: Elsevier, 2016:119—23.
4. Lin SK, Wang JT, Wu PH, et al. Apical periodontal cyst: a clinicopathologic study of 405 cases. *Chin J Oral Maxillofac Surg* 1993;4:106—19.
5. Nair PN. On the causes of persistent apical periodontitis: a review. *Int Endod J* 2006;39:249—81.
6. Hirshberg A, Tsesis I, Metzger Z, Kaplan I. Periapical actinomycosis: a clinicopathologic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;95:614—20.
7. Happonen RP. Periapical actinomycosis: a follow-up study of 16 surgically treated cases. *Endod Dent Traumatol* 1986;2:205—9.
8. Jeansonne BG. Periapical actinomycosis: a review. *Quintessence Int* 2005;36:149—53.
9. Asgary S, Talebzadeh B. Surgical endodontics for management of progressive actinomycosis. *Gen Dent* 2020;68:61—4.
10. Siqueira Jr JF, Rôças IN, Ricucci D, Hülsmann M. Causes and management of post-treatment apical periodontitis. *Br Dent J* 2014;216:305—12.
11. Wong VK, Turmezei TD, Weston VC. Actinomycosis. *BMJ* 2011;343:d6099.