

Histobacteriologic Conditions of the Apical Root Canal System and Periapical Tissues in Teeth Associated with Sinus Tracts

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Abstract

Introduction: This histobacteriologic study described the pattern of intraradicular and extraradicular infections in teeth with sinus tracts and chronic apical abscesses. **Methods:** The material comprised biopsy specimens from 24 (8 untreated and 16 treated) roots of teeth associated with apical periodontitis and a sinus tract. Specimens were obtained by periradicular surgery or extraction and were processed for histobacteriologic and histopathologic methods. **Results:** Bacteria were found in the apical root canal system of all specimens, in the main root canal (22 teeth) and within ramifications (17 teeth). Four cases showed no extraradicular infection. Extraradicular bacteria occurred as a biofilm attached to the outer root surface in 17 teeth (5 untreated and 12 treated teeth), as actinomycotic colonies in 2 lesions, and as planktonic cells in 2 lesions. Extraradicular calculus formation (mineralized biofilm) was evident in 10 teeth. **Conclusions:** Teeth with chronic apical abscesses and sinus tracts showed a very complex infectious pattern in the apical root canal system and periapical lesion, with a predominance of biofilms. (*J Endod* 2017; ■:1–9)

Key Words

Apical periodontitis, biofilm, chronic apical abscess, endodontic infection, extraradicular infection, sinus tract

Significance

All necrotic teeth associated with a periapical radiolucency and a sinus tract had bacterial infection in the apical root canal system, always organized as biofilms. In most cases, infection extended to the extraradicular environment, usually in the form of biofilms adhering to the root surface but also as actinomycotic colonies and/or planktonic bacterial cells in the lesion body.

Chronic apical abscess (also known as suppurative apical periodontitis) is a variation of apical periodontitis caused by root canal infection that resulted in an abscess draining to the surface (1). The condition is mostly characterized by a periapical radiolucency associated with an intraoral or extraoral sinus tract, which is a hallmark of the chronic abscess. The sinus tract represents a route of drainage of the abscess that follows a path of least resistance through bone, periosteum, and mucosa/skin. The chronic apical abscess is usually asymptomatic, except when the sinus tract pathway becomes closed.

Histopathologic analysis of chronic apical abscesses usually reveals a granulomatous lesion containing areas of liquefactive necrosis with disintegrating polymorphonuclear neutrophils (PMNs) surrounded by macrophages and normal PMNs (2). The sinus tract may be lined by the epithelium in its entire extent, but in the large majority of cases the epithelium does not extend deeper from the surface opening (3). In these cases, the tract pathway is mostly lined by an inflamed connective tissue (3–7).

The prevalence of sinus tracts in teeth with apical periodontitis lesions ranges from 8.5%–18% (8, 9). They are more common in teeth with large lesions (>5 mm in diameter) and frequently open intraorally on the buccal mucosa (3, 8). Sinus tracts are generally associated with longstanding infectious processes. In a study in monkeys, Valderhaug (6) extirpated the dental pulps and left the teeth open for different time periods. Sinus tracts did not develop before 100 days; they were observed in teeth that remained open for longer than 100 to 200 days. Moreover, epithelial lining of the tract was only found in late periods (6).

Endodontic infections are usually restricted to the root canal system (10). However, in some cases, bacterial infection may extend to the periradicular tissues and cause an extraradicular infection, which is usually associated with symptoms and/or persistent apical periodontitis (11–14). A study took samples from sinus tracts and reported that they always contained bacteria; in most cases, the species present in the root canal also occurred in the sinus tract (15). This is highly suggestive of an extraradicular infection. Given its pathogenesis and the reports for acute abscesses (16), chronic apical abscesses are expected to be highly associated with extraradicular infections. Bacterial infection outside the root canal system is usually characterized by the

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Clinical Research

presence of a biofilm adhering to the outer root surface or cohesive actinomycotic bacterial colonies present within the lesion body (17).

Histobacteriologic analysis permits for determination of the spatial location and organization of bacteria in an infected site. There are some histobacteriologic reports showing the association of a sinus tract and extraradicular infections, but not all cases of sinus tracts were found to exhibit bacteria beyond the confines of the canal system (10). So far, no study has focused on a consistent morphologic description of the pattern of intraradicular and extraradicular infections in teeth with sinus tracts. Therefore, the present study was conducted to perform a histobacteriologic analysis of apical root and lesion specimens taken from individuals with chronic apical abscesses and sinus tracts.

Materials and Methods

Clinical Specimens

The study material comprised biopsies of human apical peri-odontitis lesions and apices of untreated (8 cases) and root canal-treated teeth (16 cases), all of them exhibiting a sinus tract (20 actively draining and 4 apparently inactive). Biopsies originated from 24 patients (9 females and 15 males) aged 11–75 years (mean = 43 years). The medical history was noncontributory. Twelve cases were obtained by extraction because the tooth was deemed non-restorable or the patient did not agree to saving the tooth. The other 12 cases were obtained with periradicular surgery, which was performed because there was no response to nonsurgical root canal treatment and the sinus tract persisted over successive follow-ups. Periradicular surgery was performed as follows. After elevation of a full-thickness periosteal flap, the buccal bone covering the lesion was carefully removed until the pathologic tissue and the root tip were exposed. The root tip was first resected approximately 3 mm short of the apex with a fissure bur. Subsequently, the soft tissue was carefully enucleated from the bone crypt with smooth microelevators in an attempt to obtain the resected root tip and the surrounding pathologic soft tissue in 1 piece. This was accomplished when the lesion was attached to the root tip with a sleeve of fibrous tissue. In 14 of the 24 cases, the periapical lesion specimens were obtained attached to their apices in the original spatial relationship; in the other 10 cases, the lesions did not remain attached to the apices, were removed by curettage, and were processed separately.

The specimens were obtained consecutively in a dental practice operated by a single clinician over a period of 18 years (from 1999–017). The patients had given consent for examination of their teeth. Clinical and radiographic data were collected, and the occurrence of previous acute episodes was recorded. Accordingly, 11 cases had a history of an acute apical abscess in the involved teeth characterized by pain and swelling. Symptoms on the day of specimen attainment (extraction or periradicular surgery) were categorized as absent, mild, moderate, or severe. The mean diameter of the periapical radiolucency was measured, and lesions were classified as small if they were ≤ 5 mm (7 cases), large if they were > 5 mm (8 cases), or too large if they were > 10 mm (9 cases). Teeth were not included in the analysis if there was an endoperiodontal lesion or a longitudinal root fracture. Treated teeth were judged as having adequate root canal treatments on the basis of the apical terminus of fillings (not more than 1 mm short of the apex), the density of the fillings (no voids), and a tapered canal shape as determined radiographically.

Histobacteriologic Analysis

Specimens were fixed in 10% buffered formalin for at least 48 hours. With the exception of apical periodontitis lesions obtained

separately from their apices, demineralization was performed in a solution of 22.5% (vol/vol) formic acid and 10% (wt/vol) sodium citrate for a period of 3 to 4 weeks. The end point was determined radiographically. At the end of the demineralization process, specimens were washed in running water for 24 hours and dehydrated in ascending grades of ethanol. After clearing in xylene, they were infiltrated and embedded in paraffin. Next, the biopsies were oriented parallel to the long axis of the main root canal in the apical third in order to obtain sections with the canal and periapical tissue in direct continuity. Serial sections were taken with the microtome set at 4–5 μ m from the area covering the root and the surrounding lesion; the lesion periphery was not included. Every 5th slide was stained with hematoxylin-eosin for screening purposes in order to locate the areas with the most severe reactions. Selected slides were stained with the Masson trichrome to identify collagen and the Taylor-modified Brown-Brenn stain for the presence of bacteria (18).

Sections were examined for the following aspects:

1. The presence and location of bacteria in the apical portion of the root canal system, including the main canal lumen and walls and apical ramifications (intraradicular infection), within the body of the periapical lesion or adhering to the outer apical root surface (extraradicular infection): bacterial arrangements were categorized as planktonic cells or colonies or biofilm communities. The following parameter used for biofilm classification was as defined elsewhere: “populations of microorganisms that are concentrated at an interface and typically surrounded by an extracellular polymeric substance matrix” (19). Occasional bacterial cells or aggregations observed on the peripheral collagenous surface of the periapical lesion were regarded as contaminants, possibly resulting from the passage of the specimen through the socket during extraction; as such, they were excluded from evaluation.
2. The presence and distribution of acute and chronic inflammatory cells and an epithelium in the inflamed periapical tissues: lesions were diagnosed histologically as an abscess, granuloma, or cyst. Abscesses and granulomas were subclassified as epithelialized or nonepithelialized and cysts as true, bay, or unclassified. Unclassified cysts were those exhibiting an epithelium-lined cavity, but because some lesions did not remain attached to the root tip and were removed separately, it was not possible to determine whether the cavity was contiguous to the root canal (bay cyst) or not (true cyst). Unclassified cysts were also those cystic lesions for which, given the presence of a high number of apical ramifications, it was not possible to ascertain contiguity between the cyst lumen and the root canal space.

Statistical Analysis

Statistical analysis was performed to evaluate if the occurrence of an extraradicular biofilm or calculus formation was associated with any other variable. The normality of the quantitative variables was checked using the Kolmogorov-Smirnov test and graphic analysis. The continuous variable (age) was described as a mean (standard deviation) and median (range), whereas the categoric variables (sex, sinus tract activity, symptoms, radiographic lesion size, canal condition [treated/untreated], previous acute abscess episodes, and histopathologic diagnosis) were described as frequency. The difference between groups was compared using the Mann-Whitney *U* test for continuous variables and the Fisher exact test for categoric variables. The level of significance was set at 5% ($P < .05$). Statistical software (Statistical Package for the Social Sciences 21.0; IBM, Armonk, NY) was used for all analyses.

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