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Correlation between crestal alveolar bone loss with intracanal bacteria and apical lesion area in necrotic teeth

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ABSTRACT

Objective: This study aimed to analyze the correlation between crestal alveolar bone loss with the presence of some bacterial species in root canals and the apical lesion area of necrotic teeth.
Design: Data from 20 patients with diagnosis of pulp necrosis and acute apical abscesses, without active periodontal diseases, were evaluated. Patients with history of antibiotic usage three months prior to the study, with exposed pulp cavity, and with probing depth > 3 mm were not included. The root size, the distance between the bone crest to the tooth apex in the mesial and distal surfaces, and the apical lesion area were measured from standard periapical radiographies by a calibrated examiner. Root canal samples were collected using sterilized paper points. In multirooted teeth, the largest root canal was sampled. Culture, microbial isolation and identification by phenotypic methods were performed. Spearman correlation and exact Fischer test were calculated between higher/lower existing bone crests, according to the median and the presence of specific bacteria.
Results: No statistically significant differences were found between occurrence of pathogenic bacteria, such as *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, and *Prevotella intermedia*, and groups with higher/lower degree of bone loss ($p > 0.05$). A negative significant correlation was found between *Parvimonas micra* and periodontal bone loss ($p = 0.02$). Additionally, no statistically significant association was found between crestal bone loss and the apical lesion area.
Conclusions: It was concluded that, in patients without active periodontitis, the presence of pathogenic bacteria in the root canal was not correlated with periodontal bone loss.

1. Introduction

Microorganisms are the main etiological agents of both endodontic and periodontal diseases (Loe, Theilade, & Jensen, 1965; Sundqvist, 1992). These microorganisms are organized into complex communities attached to a surface and protected by a slimy layer constituted of a matrix of polysaccharides and proteins, forming a biofilm (Siqueira & Rôças, 2008). The biofilm community provides the microorganism with a series of advantages compared to their planktonic forms, such as protection against antagonic microorganisms, host defenses, antimicrobial agents, and environmental stress (Marsh, 2005).

Although these infections can be modulated by chemical and physical factors, inducing inflammation in their surrounding tissues, each component of the biofilm, such as specific bacterial species, are essential for the establishment and progression of these diseases (Hajishengallis & Sahingur, 2014; Kakehashi, Stanley, & Fitzgerald, 1965). Periapical and periodontal diseases are infections that provide an environment of nutritional diversity, which partially explains the

Abbreviations: BC-TA, crestal bone to tooth apex; BHI, Brain Heart Infusion; CEJ, cemento-enamel junction; FAA, Fastidious Anaerobe Agar; FAB, Fastidious Anaerobe Broth; ICC, intra-class correlation coefficient; Ig, immunoglobulin; IL, interleukin; RANK-L, receptor activator of nuclear factor kappa-B ligand; VMGA, Viability Medium Götemborg Agar

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polymicrobial infections. In subjects with chronic periodontitis or primary endodontic infections, strict anaerobic species are predominant (Gomes, Drucker, & Lilley, 1994; Socransky & Haffajee, 2005). Some specific examples of endodontic pathogens present in microbial communities associated with primary intraradicular infections are *Prevotella intermedia*, *Prevotella denticola*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Treponema denticola*, and *Parvimonas micra* (Conrads, Gharbia, Gulabivala, Lampert, & Shah, 1997; Narayanan & Vaishnavi, 2010; Shah & Collins, 1990). Regarding chronic periodontitis, important putative pathogens include *T. forsythia*, *P. gingivalis*, *T. denticola*, *Aggregatibacter actinomycetemcomitans* and other bacterial species (Socransky & Haffajee, 2005).

Although no study has shown an association between inflammatory markers in periodontal and endodontic lesions, it has been shown that, when analyzed separetely, both pathologies increase the levels of inflammatory markers. For instance, the serum quantity of immunoglobulin (Ig) G antibody is increased in subjects with periodontitis (Murayama et al., 1988). Additionally, one systematic review showed that the systemic levels of IgA, IgG, IgM and interleukin (IL)-1, IL-2, IL-6 were increased in subjects with apical periodontitis when compared with healthy patients (Gomes et al., 2013). These results suggest that systemic imune responses are not restricted to periodontal or to endodontic tissues, leading to increased systemic inflammation in both pathologies.

Furthermore, periodontal tissue breakdown is strongly associated with bacterial products and also with host factors, such as cytokines and cellular infiltrates (Hajishengallis & Lambris, 2012). Despite the close relationship between endodontic and periodontal diseases, studies assessing the correlation between intraradicular bacterial species and periodontal breakdown are scarce (Ma et al., 2017; Ruan et al., 2015). This study aimed to analyze statistically the correlation between crestal alveolar bone loss with the presence of bacterial species in root canals and with the apical lesion area in necrotic teeth.

2. Materials and methods

This study is a secondary analysis of a larger study, which was approved by the Institutional Review Board and by the Ethics Committee in Research of Piracicaba Dental School (State University of Campinas – UNICAMP, Piracicaba, São Paulo, Brazil) under the protocols 009/2007 and 055/2008. All individuals signed an informed consent.

2.1. Sample selection

All patients were selected from the urgency service of the Piracicaba Dental School (State University of Campinas – UNICAMP, Piracicaba, SP, Brazil). Twenty patients with pulp necrosis (determined by sensitivity tests), presence of secondary acute apical abscesses (phoenix abscess), and previous chronic apical periodontitis were included. All patients presented a slightly widened periodontal ligament to apical radiolucency, as determined by preoperative radiographic examination. All sampled teeth had the following clinical characteristics: spontaneous pain, necrosis, and pain to vertical percussion, as previously established in the literature (Gutmann, Baumgartner, Gluskin, Hartwell, & Walton, 2009). Patients with history of antibiotics usage three months prior to the study and teeth with pulp exposure to the oral cavity and probing depth ≥ 3 mm were not included. Patients with impossibility of tooth isolation and previous endodontic manipulation were also excluded.

2.2. Radiographic measurement

The methods for radiographic measurement were adapted from another study (Adyani-Fard, Kim, & Eickholz, 2011). All teeth eligible for inclusion received a standardized periapical radiography, performed using radiographic positioners. Periapical radiographs and an endodontic ruler were scanned together, using a computer program and a flatbed scanner, and then computer measured. The software ImageJ® (National Institutes of Health, Bethesda, MD, USA) was used for measurements. The endodontic rule served as a parameter for converting the measurements into real size. Root size (distance from the cementum-enamel junction – CEJ – to the apex) and the distance from crestal bone to tooth apex (BC-TA) were measured in all mesial and distal aspects of the selected tooth by one calibrated examiner (FWMGM).

For every measurement, the percentage of remaining bone was calculated by dividing BC-TA per root size and then multiplying by 100. The mean of the two measurements corresponded to the average percentage of the existing bone in each tooth.

Through the circumference of the apical lesion, the area of the apical lesion of each tooth was also measured. In multirooted teeth, only the larger apical lesion was considered for the analysis. All radiographs were digitized with a resolution of 300 dpi by a flatbed scanner with a transparency module (Printer HP Deskjet F4480, HP®, California, USA).

2.3. Microbiological sample collection

The sample collection was adapted from the protocol previously described by Gomes et al. (2004). Briefly, to collect the sample from the root canals, the tooth was locally anesthetized and then all caries and defective fillings were removed. The selected tooth received a coronary polishing and rubber dam isolation. The operative field was disinfected with oxygen peroxide 30v for 30 s, followed by 5.25% NaOCl solution for 30 s, and then neutralized with 5% sodium thyossulfate. Sterility control of the operative field was checked with a sample collection using a sterile swab. Pulp cavity access was performed with a diamond bur under copious irrigation with 0.9% sterile saline.

Samples of the root canal were collected by sterilized #20 paper points, and in multirooted teeth, sampling was performed in the largest root canal, confining the microbiological sample to a single environment. Each paper point was introduced to the approximate total length of the root canal according to a previous radiograph, remaining in such position for one minute (three paper points per canal). Continuous nitrogen flow was used to preserve the viability of the strict anaerobes. After that time, the paper points were removed from the root canal and immediately placed into plastic tubes (Eppendorf, Hamburg, Germany) containing 1 mL of VMGA III (Viability Medium Götemborg Agar) transport medium.

2.4. Laboratorial procedures for bacteria identification

Microbiological processing was performed up to four hours after the clinical procedure, according to an adaptation to Gomes et al. (2004). Inside the anaerobic chamber (Don Whitley, West Yorkshire, United Kingdom), the collected sample was shaken thoroughly in a mixer for 60 s (Vortex, Marconi, São Paulo, SP, Brazil) to facilitate microbial dispersion. After that, serial 10-fold dilutions were made up to $1{:}10^5$ in tubes containing Fastidious Anaerobe Broth (FAB – Lab M, Bury, UK). Fifty µl of each serial dilution were plated in selective culture media containing Fastidious Anaerobe Agar (FAA – Lab M, Bury, UK) + 5% defibrinated sheep blood + hemin (1 ml/l) + menadione (1 ml/l). These samples were incubated in an anaerobic chamber at 37 °C in an atmosphere of 10% H_2, 10% CO_2 and 80% N_2 for 14 days, as an attempt to allow detection of strains that are very slow-growing.

The same dilutions were also plated in Brain Heart Infusion (BHI) agar (Oxoid, Basingstoke, UK), supplemented with defibrinated sheep blood, and incubated aerobically at 37 °C for two days to allow aerobic or facultative microorganism growth.

The incubation periods and conditions for each culture were done as follows: