Prevalence of microorganisms in root canals of human deciduous teeth with necrotic pulp and chronic periapical lesions

Prevalência de microrganismos em canais radiculares de dentes deciduos de humanos com necrose pulpar e lesão periapical crônica

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ABSTRACT: The objective of this study was to evaluate bacterial prevalence in 31 root canals of human deciduous teeth with necrotic pulp and periapical lesions using bacterial culture. After crown access, the material was collected using absorbent paper points for microbiological evaluation and determination of colony forming units (CFU). Anaerobic microorganisms were found in 96.7% of the samples, black-pigmented bacilli in 35.5%, aerobic microorganisms in 93.5%, streptococci in 96.7%, and S. mutans in 48.4%. We concluded that in human deciduous teeth root canals with necrotic pulp and periapical lesions the infection is polymicrobial, with a large number of microorganisms and a predominance of streptococci and anaerobic microorganisms.

DESCRIPTORS: Tooth, deciduous; Bacteriological techniques; Periapical abscess.

INTRODUCTION

Although oral health education and prevention are priorities in contemporary dentistry, pulp changes in deciduous teeth are very frequent. Thus, curative dentistry should simultaneously evolve and improve concepts and therapeutic procedures.

Despite the controversy, many authors report the need to treat root canals of deciduous teeth with pulp necrosis5,6,12 due to the propagation of microorganisms throughout the entire root canal system, including the lumen, lateral, accessory and secondary canals, dentinal tubules, ramifications of the apical delta, apical foramen, areas of apical cementum resorption and periapical biofilm7,11.

The success of endodontic treatment depends on many factors and the reduction or elimination of bacterial infection5,11 is the most important one. However, for this to occur, it is important to identify which microorganisms are present. Little research has been done to identify which bacterial species are present5,6,10,13,20,21 in deciduous teeth with pulp necrosis and periapical infection.

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Therefore, the aim of this study was to evaluate the bacterial profile in root canals of human deciduous teeth with necrotic pulp and periapical lesions.

**MATERIAL AND METHODS**

**Clinical procedures**

The research protocol was approved by the Institution’s Ethics Committee (#2000.1.488.58.7).

Fifteen 3- to 7-year-old children, of both sexes, seen at the Pediatric Dental Clinic, School of Dentistry of Ribeirão Preto (University of São Paulo) were selected. None of the children had been treated with antibiotic for at least 3 months. A total of 31 root canals from 18 deciduous teeth (maxillary incisors and canines, and maxillary and mandibular molars) with necrotic pulp and radiographically visible radiolucent areas in the region of the bone furcation and/or the periapical region suggesting chronic periapical lesion were used. The teeth had carious lesions, some with the pulp chamber exposed to the oral cavity. However, it was possible to isolate the surgical field with a rubber dam and restore the tooth. They also had intact roots or less than 2/3 of physiological root resorption, as determined by odontometry 1 mm before the radiographic apex or the limit of the physiological root resorption. The teeth were treated for the immediate and progressive neutralization of septic/toxic content using K-files and copious irrigation/suction with 2.5% sodium hypochlorite followed by odontometry 1 mm before the radiographic apex or the limit of the physiological root resorption. Biomechanical preparation was carried out with sequential K-files and irrigation with 2.5% sodium hypochlorite. The canals were subsequently dried with sterile absorbent paper points and filled with EDTA (Odahcam Herpo Produtos Dentários Ltda., Rio de Janeiro, RJ, Brazil), and mixed for 3 min with a K-file to remove smear layer. The canals were irrigated and dried, and then filled with calcium hydroxide paste (Calen PMCC, S.S. White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil) with a special syringe (ML, S.S. White Artigos Dentários Ltda., Brazil). The pulp chamber was sealed with zinc oxide/eugenol cement (IRM, Dentsply Indústria e Comércio Ltda., Petrópolis, RJ, Brazil). After 14-30 days, the intracanal dressing was removed and the canals were filled with Calen thickened with zinc oxide, as recommended by Silva and Leonardo, and restored.

**Microbiological processing**

The tubes containing the samples were mixed with 4-6 sterile glass beads in a mixer (Mixtron-Toptonix, SP, Brazil) for 2 min at maximum speed. Subsequently, serial decimal dilutions up to $10^{-5}$ were made in Sorensen Phosphate Buffer (PBS) under laminar airflow. A volume of 0.05 ml of the pure samples and of each dilution was sown, with a sterile calibrated pipette, onto plates containing As (Blood agar, Difco, Detroit, USA), Ms (Mitis...
Salivarius agar, Difco, Detroit, USA) and Ask media (Blood agar supplemented with hemin and menadione, Sigma Chemical Co., St. Louis, USA). Plates containing SB\textsubscript{20} agar (Bacitracin sucrose agar, Difco, Detroit, USA) received dilutions only up to 10\textsuperscript{4}. The samples were then distributed uniformly from the most diluted to the least diluted using a sterile glass L-shaped rod.

The Ask plates were incubated anaerobically using the Gas-Pak system, in hermetically sealed jars (Permution, Equipamentos e produtos quimicos Ltda., Curitiba, Brazil) for 7-10 days. The As plates were incubated aerobically for 24-48 h, at 37\textdegree C, and the Ms and SB\textsubscript{20} plates were incubated for 3 days in microaerofic conditions (candle jar system) in a hermetically sealed jar. After incubation, colonies were counted with a stereomicroscope (Nikon, Tokyo, Japan) under reflect light and the colony forming units (CFU/ml) were calculated.

From the SB\textsubscript{20} agar plates, 3-4 colonies suspect of being *mutans* streptococci were isolated and identified according to Ito et al\textsuperscript{4}: fermentation of mannitol, sorbitol, raffinose and melibiose; hydrolysis of arginine and sculin; and sensitivity to bacitracin.

**RESULTS**

The CFU (colony forming units) of the samples from 31 root canals from deciduous teeth with necrotic pulp and radiographically visible periapical lesion are presented in Table 1.

The prevalence of anaerobic microorganisms was 96.8% (30 root canals), and black-pigmented bacillus (BPB) were found in 11 cases (35.5%). Aerobic microorganisms were present in 29 root canals (93.5%) with streptococci present in 30 canals (96.8%). *Streptococcus mutans* was quantified in 15 canals (48.4%) whereas *Streptococcus sobrinus* was not detected.

**DISCUSSION**

During the 1950s and 1960s, researchers isolated mainly aerobic and facultative bacterial species from root canals with necrotic pulp and periapical lesions due to the limitation of isolation techniques and microbial culture\textsuperscript{14}. With scientific and technological evolution, anaerobic techniques have been developed and, in the 1980s, this concept was modified showing that in root canals of permanent teeth with necrotic pulp and periapical lesions there is a polymicrobial infection with predominance of strict anaerobic species\textsuperscript{1,17,22}.

The microbiota is constituted of only a few species when compared to the total bacteria of the oral cavity. There are many factors that can influence the growth and development of these microorganisms in root canals, i.e., nutrient availability, low oxygen tension, bacteria interaction, as well as disintegrated pulp tissue and tissue fluids that are essential nutrient sources\textsuperscript{17}.

In the present study, anaerobic microorganisms were detected in 96.8% of the samples. These results are in agreement with those of Toyoshima et al\textsuperscript{21}, Sato et al\textsuperscript{13} and Faria\textsuperscript{6}, who reported that in root canals of primary teeth with periapical lesions there is a polymicrobial infection with predominance of anaerobic microorganisms, similar to those of the microbiota of permanent teeth.

Black-pigmented bacilli (BPB) have frequently been isolated from root canals of permanent teeth with necrotic pulp. Sundqvist et al\textsuperscript{18} reported their presence in 30% of the cases while Assed et al\textsuperscript{1} verified by immunofluorescence that these microorganisms were found in 60% of the samples. In the present study in deciduous teeth, BPB were found in 11 cases (35.5%), a figure which is similar to those found in reports by Tomic-Karovic, Jelinek\textsuperscript{20} and Faria\textsuperscript{6} who detected these microorganisms in 36% and 30%, respectively, of the root canals of deciduous teeth with necrotic pulp. However, Toyoshima et al\textsuperscript{21} quantified BPB in 44.4% of deciduous root canals in retreatment cases.

Studies of permanent teeth associate the presence of BPB with the development of abscesses\textsuperscript{18}. We agree with Faria\textsuperscript{6} who did not observe this correlation when considering deciduous teeth. In the present study, 8 of the 19 root canals with fistulae had BPB. However, this microorganism was also detected in 3 cases in which there were no fistulae.

In this study, aerobic microorganisms were quantified in deciduous teeth with necrotic pulp and chronic periapical lesions in 29 root canals (93.5%). Although Sato et al\textsuperscript{13} and Faria\textsuperscript{6} observed a higher prevalence of anaerobic microorganisms over aerobic microorganisms, in our study prevalence rates were similar (93.5% aerobic and 96.8% anaerobic). These results show that endodontic infections in deciduous teeth, similarly to those in permanent teeth, are polymicrobial with the development of microbial interactions.

The literature shows the presence of streptococci in 70\textsuperscript{6}, in 82\textsuperscript{19}, in 76\textsuperscript{20} and in 85\textsuperscript{6} of the root canals of deciduous teeth with pulp necrosis. In our study, streptococci were detected in 30 cases (96.8%). Streptococci and anaerobic microorganisms were the most prevalent bacteria in the
deciduous teeth with necrotic pulp and periapical lesions.

*Mutans* streptococci were found in 15 root canals (48.4%); however, only *S. mutans* was found. *S. sobrinus* was not found in any case. Regarding the quantification of *mutans* streptococci in root canals of permanent teeth, many studies have detected this microorganism, which was found by Stobbering, Eggink in 3.05% of the cases, by Baumgartner, Falkler Jr. in 33.4%, and by Assed et al. in 52.0% of the cases. However, in deciduous teeth root canals only Faria reported the presence
of mutans streptococci (30.0%) in which S. mutans was found in 25.0% and S. sobrinus in 5.0% of the cases. The variations in the prevalence of these microorganisms in different studies can be explained by the fact that in the cases in which the numbers were higher, some root canals could have been exposed directly to the oral cavity, enhancing the prevalence of S. mutans, as observed in our study.

According to the results of this study, anaerobic microorganisms, black-pigmented bacilli, aerobic bacteria, streptococci and mutans streptococci are components of root canals of deciduous teeth with periapical lesions. Because of the importance of microorganisms in the etiology of pulp and periapical changes, the lack of in vitro and in vivo studies evaluating the root canal microbiota of deciduous teeth with different degrees of pulp and periapical pathoses is not understandable. Thus, there is no consensus on which technique and material is better for the reduction and/or elimination of these microorganisms, so that the deciduous teeth can remain in function in the oral cavity until exfoliation. Therefore, we suggest further research on this subject.

CONCLUSIONS

According to the obtained results, we can conclude that, before biomechanical preparation, the root canals of deciduous teeth with necrotic pulp and chronic periapical lesion presented a high number of microorganisms and polymicrobial infection with the prevalence of anaerobic bacteria and streptococci.

REFERENCES


