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Microbiologic endodontic status of young traumatized tooth

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Abstract –Traumatic dental injuries could expose the dentin and, even the pulp, to the oral environment, making possible their contamination. The presence of microorganisms causes pulpal disease and further a tecidual clutter in the periradicular region. The therapy of periradicular pathosis is the consequence of a correct diagnoses which depends on the knowledge of the nature and complexity of endodontic infections. As there is no information on the microbiology of primary endodontic infection in young teeth, the aim of the current study was to investigate the microbiologic status of root canals from permanent young teeth with primary endodontic infection. Twelve patients with the need for endodontic treatment participated in the study. The selected teeth were uniradicular and had an incomplete root formation. They had untreated necrotic pulp. After the access preparation, nineteen microbiologic samples were obtained from the root canals with sterile paper points. Afterwards, the paper points were pooled in a sterile tube containing 2 ml of prereduced transport fluid. The samples were diluted and spread onto plates with selective medium for *Enterococcus* spp. and for yeast species and onto plates with non-selective medium. A quantitative analysis was performed. The mean number of cultivable bacterial cells in the root canals was 5.7×10^6. In four samples (21.05%) black pigmented species were recovered and the mean number of cells was 6.5×10^5. One specimen (5.25%) showed the growth of *Enterococcus* species and the mean number of cells in this case was of 1.5×10^4. The results showed a root canal microbiota with similar design as seen in completely formed teeth.

When dental pulp is exposed because of traumatic fractures or cracks, oral bacteria can invade and cause pulp inflammation (1). Metabolic and other toxic products of these bacteria are thought to be responsible for such inflammatory reactions and subsequent disintegration and infection of pulp space. If infection in the canal persists, chronic inflammation of the periapical area ensues leading to bone loss (2).

All bacteria within the oral cavity share the same opportunities for invading the root canal space; however, only a restricted group of species has been identified in infected root canals. The reason for the disproportionate ratio between potential and actual number of species is that the root canal is a unique environment where biologic selection drives the type and course of infection. An anaerobic milieu, interactions between microbial factors and the availability of nutrition are principal elements that delineate the composition of the microbial flora (1).

Abundant scientific evidence supporting the microbial etiology of periradicular diseases has been accumulated. Earlier studies of the endodontic microbiota indicated that aerobic and facultative bacteria are dominant isolates. However, the improvements in anaerobic culturing techniques during the last 20 years have established that obligately anaerobic bacteria predominate in infected root canals (3–7).

The number of bacterial species in an infected root canal may vary from one to more than 12 and the number of bacterial cells varies from $< 10^2$ to $> 10^8$ per sample. A correlation seems to exist between the size of the periapical lesion and the number of bacterial species and cells in the root canal. Teeth with long-standing infections and large lesions usually harbor more bacterial species and have a higher density of bacteria in their root canals than teeth with small lesions (2).

The knowledge about the patterns of microbial organization in root canal infections assumes special importance in both the understanding of the disease process and the establishment of an effective antimicrobial therapeutic strategy (8, 9).

Nevertheless, early identification studies do not give information concerning the patterns of microbial colonization of the root canal system of young teeth where anatomic, histologic and morphological differences could affect the characteristics of the infectious process.

Thus, considering the importance of microorganisms in pathogenic processes involving the root canal system, the purpose of this study was to provide clue information with regard to the microbiologic characteristics of the

primary endodontic infection in root canals from permanent young teeth.

Materials and methods

Patient selection

Twelve patients who had been referred to the Traumatism Service – CADE – Trauma – at the Dental School of São Paulo University, SP, Brazil, were included in the study. The age of the patients ranged from 7 to 11 years. One patient, who had trauma many years ago, was 22 years. The selected teeth (one or more per patient) were single rooted with an incomplete root formation, their pulp chamber was without visual communication with the oral fluid, they presented with necrotic pulp tissue and showed radiographic evidence of periapical periodontitis, but an absence of periodontal disease. A detailed medical and dental history was obtained from each patient. Subjects who had received antibiotic treatment during the previous 3 months or who had a general disease were excluded from the study. All patients had been informed about the study and had given their informed consent. The study was conducted in full accordance with the declared ethical principles (World Medical Association Declaration of Helsinki, version VI, 2002) and had been approved by the Research and Ethics Committee of the Dental School of São Paulo University (160/08).

Microbiologic sampling

After local anesthesia had been administered, the tooth to be treated was isolated with a rubber dam. The tooth and surrounding dam and clamp were disinfected with 30% hydrogen peroxide and then swabbed with 1% tincture of iodine. The surface was then swabbed with 5% sodium thiosulfate solution to inactivate the iodine solution (5, 9). A control sample for the asepsis of the operation field was taken. The tooth and rubber dam were scrubbed with a sterile cotton pellet, which was then transferred to a transport medium (VMGA III). Aseptic techniques were used throughout endodontic procedures and sample acquisition. An access cavity was prepared with sterile burs under cooling with sterile NaCl. Bacterial sample was taken as soon as the pulp chamber was reached, using a sterile paper point (TANARI, Amazonas, Brasil). Three sterilized paper points were sequentially placed to reach the estimated length of the root determined with the preoperative radiograph, and kept in place for 30 s (10). In cases where a dry canal was identified a small amount of saline was used to ensure viable sample acquisition. Afterwards, the paper point sample from the root canal were pooled in a sterile tube containing 2 ml of transport medium VMGA III (Viability Médium Goteborg Anaerobically) and forwarded to the microbiology laboratory.

Microbiologic assessment

Inside the laminar flow cabinet the transport media were shaken thoroughly in a mixer for 30 s (Fisher Vortex Genie 2; Fisher Scientific Inc., Traverse City, MI, USA) (11). The transport media contained glass beads 3 mm in diameter to facilitate mixing and homogenization of the sample prior the cultivation. Serial 10-fold dilutions to 10^{-4} were made up in tubes containing peptonated water. One hundred microliters of each dilutions from 10 through 10^{-4} were plated, using sterile glass spreaders, onto plates with specific culture media for *Enterococcus* spp., m-*Enterococcus* agar (Difco Laboratories, Detroit, MI, USA); onto plates with selective medium for yeast species, Sabouraud-dextrose agar (Difco Laboratories) supplemented with 100 μg/ml of chloramphenicol (Medley, Campinas, SP, Brazil); and onto plates with non-selective medium, Brucella-blood agar (Difco Laboratories) supplemented with 5% defibrinated sheep blood, 10 mg/l hemin (Sigma Chemical, St. Louis, MO, USA) and 1 mg/l menadione (E. Merck, Darmstadt, Germany). The plates containing m-*Enterococcus* agar were incubated aerobically at 37°C for 48 h. Sabouraud agar plates were kept at room temperature for up to 5 days. Brucella-sangue agar plates were incubated at 37°C in anaerobic atmosphere (80% N_2, 10% H_2, 10% CO_2) for 14 days. After incubation time the bacterial growth was quantified by enumerating the colony-forming units on each culture plate using QUIMIS accountant of colonies (QUIMIS Diadema, São Paulo, Brazil) and the number of colony-forming units per milliliter for each dilution was calculated for each sample.

Results

All swab samples over the wall of the access cavities were negative. A total of 19 root canals were sampled and all of them contained anaerobic bacteria. Under this condition, the mean number of cultivable bacterial cells in the root canals was 5.7×10^6 ranged from1.8×10^3 to 1.15×10^7. The number of bacteria in each sample is shown in Table 1.

Table 1. Colony forming units per milliliter in each sample not diluted and diluted from 1/10 to 1/10 000

Samples	No dilution	1/10	1/100	1/1000	1/10 000	cfu's/ml
1	–	–	119	–	–	1.19×10^5
2	–	–	–	125	–	1.25×10^6
3	–	–	–	190	–	1.90×10^6
4	–	–	–	201	–	2.01×10^6
5	–	–	–	135	–	1.35×10^6
6	–	–	53	–	–	5.3×10^4
7	–	–	–	41	–	4.1×10^5
8	–	295	–	–	–	2.95×10^4
9	–	–	–	238	–	2.38×10^6
10	–	–	–	78	–	7.8×10^5
11	–	–	–	–	115	1.15×10^7
12	–	–	–	38	–	3.8×10^5
13	–	–	223	–	–	2.23×10^5
14	189	–	–	–	–	1.89×10^3
15	–	–	–	208	–	2.08×10^6
16	–	–	273	–	–	2.73×10^5
17	–	–	–	37	–	3.7×10^5
18	–	–	–	154	–	1.54×10^6
19	–	–	–	186	–	1.86×10^6

Table 2. Colony forming units per milliliter of black pigmented bacteria

Samples	No dilution	1/10	1/100	1/1000	1/10 000	cfu's/ml
9	–	–	–	85	–	8.5×10^5
10	–	–	–	53	–	5.3×10^5
11	–	–	–	127	–	1.2×10^6
14	–	–	118	–	–	1.1×10^5

Black pigmented species were recovered from 4/19 (21.05%) root canals samples. The mean number of cells per sample was 6.5×10^5 varying from1.1×10^5 to 1.2×10^6 (Table 2).

The median number of bacterial cells recovered from root canals containing black pigmented Bacteroides was 5.74×10^6 (range 1.89×10^3 to 1.15×10^7) and the median number of cells from the other 15 canals was 9.2×10^6 (range 2.95×10^4 to 2.08×10^6). One root canal (5.25%) yielded *Enterococcus* species and the number of colony-forming units was 1.5×10^4. No sample showed growth of yeast species.

Discussion

The quantitative analysis performed discloses a microbiologic design of primary endodontic infection in young teeth as similar as the one seen in completely formed teeth.

It is well established that bacteria and their by-products present in root canals are directly related to the development and maintenance of periapical lesions (4,12). The structure and the dynamics of root canal system infections have been studied along the years. The development of anaerobic techniques has led to considerable progress in clarifying the etiopathogenesis of endodontic infections and has shown that most of them are polymicrobial, with prevalence of obligate anaerobic bacteria (13). An infection is developed if the invasion of microbes produces damage to tissue. Endodontic disease (pulpal and periradicular) is the result of both the pathogenic effects of the microbes and the response of the host (14). Apical periodontitis is a dynamic process that has distinct pathogenetic stages, where species found in later stages were not necessarily present in the early stages (15).

Anaerobic conditions inside the root canal system are resulting from the changes in available nutrition as well as a decrease in oxygen availability. A decrease in availability of carbohydrates and the wide range availability of peptides and amino acids, which are the main source of nutrition for anaerobic microbes, limits growth opportunities for facultative anaerobes. At the same time, the consumption of oxygen and production of carbon dioxide and hydrogen along with the development of a low reduction–oxidation potential by the early colonizers, favor the growth of anaerobic bacteria (2, 16).

The goal of clinicians is to disrupt and destroy the microbial ecosystem associated with the disease process. No other single factor has had such importance for improving the documented rate of treatment success as the appreciation and incorporation of antimicrobial principles (17).

A clinical study in humans is a foundation for the daily practice procedures and may explain unknown factors concerning the etiology and evolution of this disease process.

The root formation comprise dentinal mineralization (18) and many studies had mentioned alterations in this process caused by evolution course (18–20). Morphologic and functionary variations take place on the pulp-dentin complex as a result of the evolution process and could inspire the microbiologic characteristics of the infection. In this study, root canal samples were obtained from young teeth referred for endodontic treatment after trauma.

The microbiologic assessment carried out recovered anaerobic bacteria from all samples. Median total bacteria counts amounted to 5.7×10^6 UFC's/ml. Similar results were described for completely formed teeth (21–23).This data disclose remarkable similarly in microbiologic characteristics in primary endodontic infections from both young teeth and completely formed teeth.

Four of 19 (21.05%) root canal samples were positive for growth of black pigmented species. These findings disagree with the results of similar investigations which found this species in high prevalence in infected root canals of completely formed teeth (24–27). There may be several explanations for the rare isolation of the black pigmented species. The growth is very slow, even on enriched media, and it requires an incubation time of 7 days or more before pigmentation is visible. Furthermore, this species is very sensitive to oxygen and could have been killed by the long time of exposure during a protract sampling procedure as the subjects had low ages.

There was no great difference in the median number of colony-forming units enumerated between root canals infected with black pigmented species (5.75×10^6) and root canals not infected with black pigmented bacteria (9.2×10^6). These results corroborate the findings of Baumgartner & Falkler (24). Nevertheless incompatible results had been described by Sundqvist, Johansson and Sjögren (27).

In this study, *Enterococcus* species were recovered from only one canal (5.25%). Recent issues related high incidence of *Enterococcus faecalis* in primary endodontic infection (21, 28, 29). This discrepancy observed may be caused by the different methodological techniques employed.

Although the role of yeasts in endodontic infection has not been established, microorganisms of the genera *Candida albicans*, present in the indigenous oral microbiota, have been recovered from root canals presenting necrotic pulp (30–32). In this study, this microorganism was not recovered from any root canal sample.

The relation between the presence of root canal infection and the development of the periradicular disorders constitutes a dogma in endodontic microbiology. This concept results from of exhausting research which had provided a better biologic agreement of the pulp and periradicular pathological alterations. In this

context one of the factors that deserves prominence would be the necessary connection between the knowledge acquired by means of research and the principles of treatment that conduct the practical clinic.

The development of effective strategies for root canal therapy is, therefore, based on information derived from studies that had delineated the standard of microbial settling of infected root canals.

Future research using diverse technical methodologies are warranted to further explore the microbiologic status of endodontic infection of young teeth.

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