Bacterial Aggregation in Infected Root Canal

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Abstract

The aim of this study was to investigate different microbial morphotypes in the root canal infection associated with chronic diffuse periapical lesion. In forty cases of asymptomatic teeth with radiographically diagnosed diffuse periapical lesion we took specimens of infected tissue from the root canals at the beginning of endodontic treatment. Fixation and four different staining methods of the specimens were obtained to provide microscope examination. All examined root canal specimens were heavily infected by bacteria. The most commonly identified were cocci 92 %, small mostly G+ diplococci and large G+cocci in clusters and grapelike groups, bacilli found in 67%, coccobacilli 37%, fungi 17%, and spirochetes in 5%.

KEY WORDS: root canal infection, bacterial morphotype, microscopic examination

Introduction

Microscopic identification of the size, shape and different morphotypes of the present bacteria in infected root canal is important because of understanding of the disease process and establishment of an effective antimicrobial strategy. Study with light microscope was also conducted to disclose different bacterial stainings. Morphologically the root canal microbiota is consisted of cocci, bacilli, coccobacilli, fungi and spirille. In 1894. WD Miller published his findings on the bacteriological investigation of many different microbes in the infected pulp space, observed by microscopic examination(1). Some microbes were uncultivable when compared with the full range observed by microscopy, and that the flora was different in the coronal, middle and apical parts of the canal system (2). Due to limitations of sampling and cultivation technique this observation was not verified until 1982 by culturing (3). More recent experiments have shown that the endodontic milieu is a selective habitat for the development of anaerobic micro-

flora. The development of specific proportion of the root canal microflora is determined by the consumption of oxygen and oxygen products. 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 <102 to >10 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 (2).

Aim

The aim of this study is to give evidence of size, and different bacterial morphotypes in infected root canal as well as their frequency in cases associated with diffuse chronic periapical lesion. Prevalence of bacteria found by microscope examination is suggestive of next more accurate microbiological procedure necessary to provide exact bacterial findings. Improvement in future endodontic therapy also depends on exact bacterial identification.

Material and Methods

SPECIMEN SAMPLING

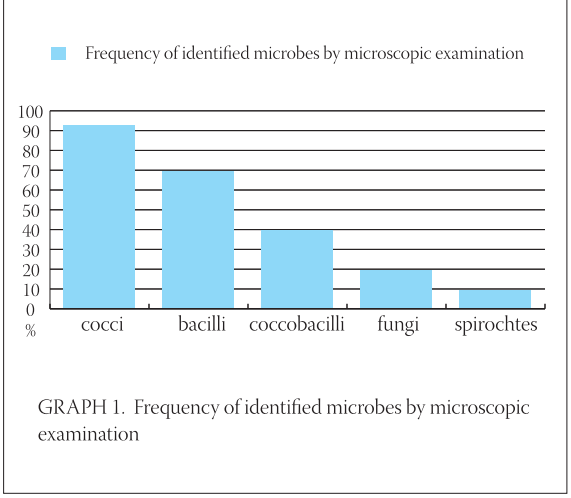
In forty cases of asymptomatic clinically and radiographically diagnosed chronic periapical lesions we took specimens of infected pulp tissue from the root canal. Samples were collected using strict asepsis. The tooth is isolated, cleansed, and decontamined with hydrogen peroxide and sodium hypochlorite solution. Access preparation made by sterile burs, restoration and carious lesions completely removed using sterile burs without water spray. If the root canal is dry, 0,5 mL of sterile saline solution should be injected before sampling. ISO 15-25 K-files used to scale off dentin debris from the root canal walls, and prepare specimens for fixation and staining. First step in the processing of most clinical material which we followed, is microscopic examination of the specimens. Information is obtained from smear preparation. To have a representative smear it must not be too thick because during staining material could easily be washed off and then uninterpretable. In order to achieve diagnosis of different bacterial morphotypes we had to do various kinds of differential staining methods. The Gram stain is particularly helpful in providing presumptive evidence of the possible presence of anaerobes because many anaerobic organisms display distinctive features on microscopic examination.

STAINING METHODS

Four different staining methods used are: Gram stain, methylene blue stain, Giemsa stain, and Warthin-Starry-Faulkner method. Gram stain method is an differential empirical staining procedure devised by Gram, in which microbes are stained with crystal violet, treated with 1:15 dilution of Lugols iodine, decolorized with ethanol and counterstained with a contrasting dye, usually safranin. Those microbes that retain the cristal violet stain are G+, and those that lose the cristal violet stain by decolorization, but stain with counterstain are G-. Methylene blue, an aniline dye used as staining agent, prepared in a saturated solution seven per cent in absolute alcohol, which is diluted for use. Giemsa stain is a differential staining, a solution containing azure II-eosin, azure II, glycerin and methanol. Stained elements appear pink to purple. Warthin-Starry-Faulkner method is a silver stain method for staining spirochetes. A smear is air dried, immersed in absolute ethanol, washed in distilled water, incubated in 2 per cent silver nitrate. The cover glass is then developed in a mixture of silver nitrate, gelatin, glycerol, agar and hydroquinone. Spirochetes appear black on a light background.

Results

All examined root canal specimens were heavily infected by bacteria. Microbial aggregates composed of one or more distinct morphologic type were observed colonizing the root canal. Bacterial colonies consisting of only one definite morphology type might have been self-aggregating bacterial forming cluster. Method used in this study does not permit species identification. Each bacterial cell observed in the root canal system could have been an endodontic pathogen.



GRAPH 1. Frequency of identified microbes by microscopic examination

Frequency of identified microbes by microscopic examination using four different staining methods: - cocci 92 %, bacilli 67 %, coccobacilli 37%, fungi 17%, spirochetes 5%, graph 1. The most commonly identified were coccci, large in clusters and grapelike groups, small, mostly diplococci arranged in pairs and chain also G+.

Discussion

Our findings correlate with more accurate microbiological findings of the specimens from infected root canal, by Gomes et al. (4), Peters et al. (5), Jacinto et al.(6), which also present cocci and bacilli as the most frequently identified microflora. The presence of G+ cocci mostly streptococci and staphylococci were found in 80% of the bacteria isolated from more than 4000 necrotic root canal tissue by Winkler and Van Amerongen. They also found the presence of G- rods. Primary endodontic infections are caused by oral microorganisms which are usually opportunistic pathogens. Any of the more than 500 microbial species from the oral microbiota may invade and colonize the pulp tissue (7). Nowadays evidence indicates that some bacterial species are related with some forms of periradicular diseases and thereby are considered putative endodontic pathogens (8). Our study showed that the root canals of teeth associated with chronic diffuse periapical lesions were heavily colonized by bacteria and rarely by fungi. Gram positive cocci appear as micrococci, spherical cocci arranged in pairs and chain (Figure 1) or arranged in tetrads and grapelike clusters (Figure 3). The Gram negative cocci occur singly or in pair's (Figure 4). The Gram negative cocobacillary rods display morphological feature from uniform to pleomorphic (Figure 5). Figure 2 presents spherical and oval shaped fungal cells. It has been noted that the endodontic flora can appear in clusters of mixed bacterial content and matrix-enclosed communities such as biofilms that evolved bacteria to permit survival of the whole community (9).

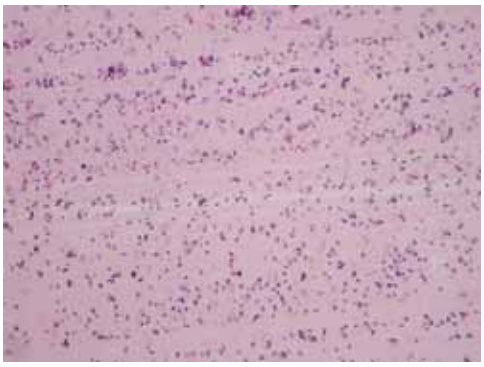


FIGURE 1. Gram stain, small cocci, diplococci G+.

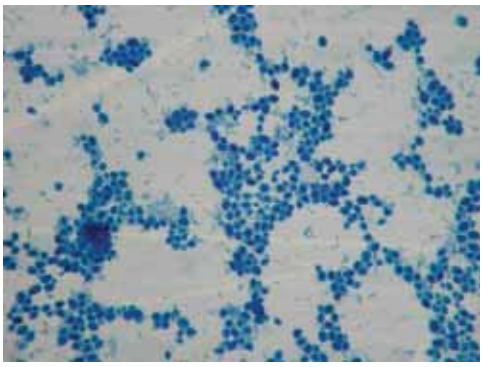


FIGURE 2. Methylene blue stain, fungi

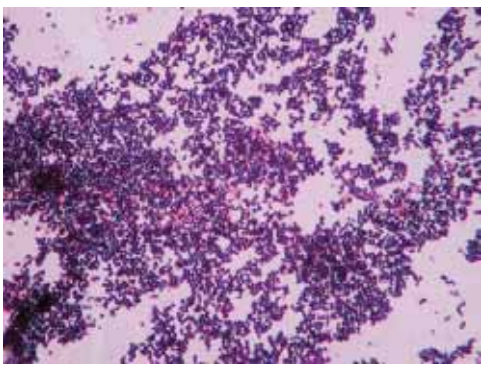


FIGURE 3. Gram stain, large cocci G+.

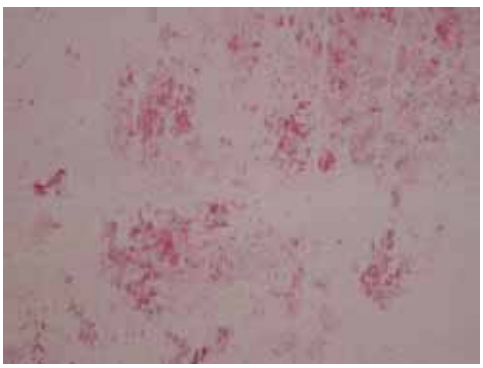


FIGURE 4. Gram stain, cocci G -.

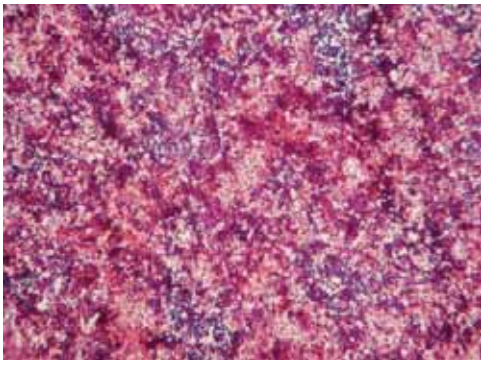


FIGURE 5. Gram stain, coccobacilli G -.

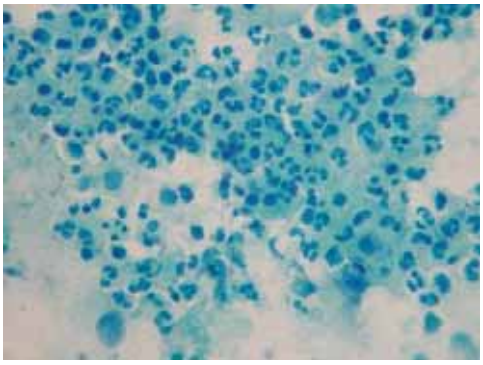


FIGURE 6. Methylene blue stain, polymorphonuclear leukocytes.

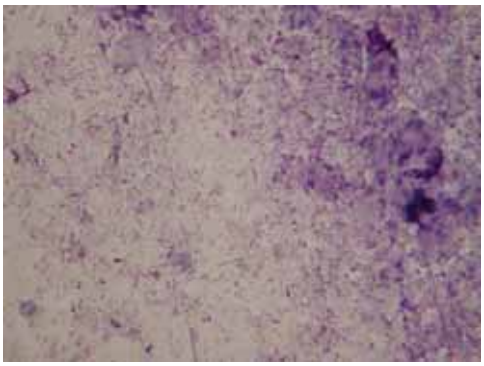


FIGURE 7. Rapid Giemsa stain, diplococci, bacilli, coccobacilli.

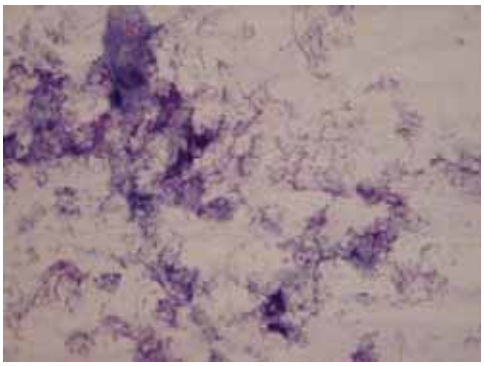


FIGURE 8. Rapid Giemsa stain, bacilli.

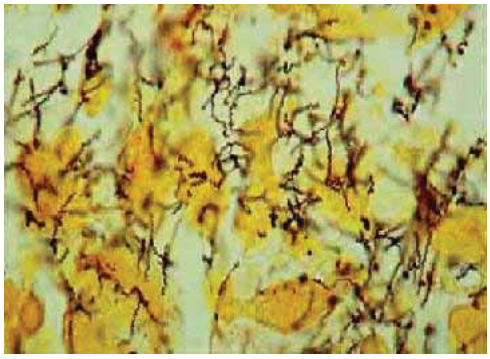


FIGURE 9. Warthin-Starry-Faulkner stain, spirochetes.

Matrix–enclosed community presents mixed bacteria (Figure 7 and 8). The Warthin-Starry-Faulkner staining method demonstrated tightly coiled spirochetes, long and very thin with characteristic motility (Figure 9). The microscopic pattern of the root canal microbiota require adequate consideration in endodontic treatment and prevention of clinical complications (10). The findings of this and other morphologic studies should be used to understand root canal infection and to establish adequate measures to completely eradicate infection.

Conclusion

The root canal infection is a dinamic process and various bacterial species dominate at different stages of the process. Tendence in a long standing infection is a dominance of the community by selected species. Factors which are driving this development are: availability of nutrition, oxygen level (redox potential) and the local pH within the root canal (2). Accumulation and co-aggregation of bacteria suggest that synergistic interaction is taking place between the organisms involving use of food chains and consorted degradation of complex host and bacterial exopolymers (11). Positive bacterial interaction seems to exist not only in the root canal but also in periapical lesion as well as in the periodontal pocket (7, 12, and 13). Microscopic presentation of the root canal bacteria is the first step in clinical presentation which provides informations that leads clinician to next more accurate diagnostic method.

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