A Microbiological Study of Periapical Lesions in Single Rooted Teeth with Open and Closed Root Canal

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Summary

The aim of this study was to investigate the microbial flora of periapical ostitic lesions in single rooted teeth. Periapical processes in which the root canal was open and those in which the canal was closed (reinforcement) were analysed. Samples for microbiological analysis were taken during the operation - apicotomy. On the whole the results obtained agree with current knowledge of the microbial flora in odontogenic infection. The polymicrobial composition of the flora with a significant share of anaerobic bacteria and reduced microbial flora in closed ostial lesions are the basic characteristics of periapical infection. The most frequent isolates found were from the streptococci group, and the most frequent anaerobe, Veillonela. It was determined that streptococci occur more frequently in closed ostial processes.

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Introduction

In many ways the oral cavity represents a specific area, the characteristics of which condition the diversity of microbial species. The environment of the oral cavity, its microclimate, diverse tissue and numerous microlocalities comprises a very favourable site for the growth of oral microflora. The microbiological population which invades the oral cavity is as varied as the substrate on which it lives. The microorganisms present in oral physiological flora are bacteria, fungi, viruses, micoplasms and protozoa (1).

Within the oral cavity are microlocalities which are mutually different according to the qualitative and quantitative composition of the microbial flora: saliva, teeth, dental plaque, gingival sulcus, cheek mucous membrane and tongue mucous membrane (2).

Maintenance of the microbial flora in the oral cavity is a dynamic and continuous process. Microbial flora changes, depending on physiological and pathological conditions through which the whole organism passes (3). The use of antibiotics may also lead to alteration of normal flora (4, 5), which can lead to susceptibility to certain infections and superinfections (6).

Many authors have carried out investigations of microbial flora in the area of the oral cavity, both physiological and pathological. However, their investigation results leave much for further study, due to the fact that the microbial flora in odonto-

genic inflammation is qualitatively and quantitatively an extremely variable entity. The results of investigations also depend a great deal on the group of subjects, the sampling method (7) and the phase of inflammation in which the sample is taken.

According to available literature, over 50 species of microorganisms have been isolated from the oral cavity, mainly bacteria which are considered the causative agents of odontogenic infections. However, this number is nowhere near the actual number of microbial flora in the oral cavity. Namely, it is considered that there are between 300 and 500 species of bacteria in the oral cavity (16). Methodology and technology of sampling, cultivation and identification are being developed which will enable new knowledge of the microorganisms in the oral cavity.

Odontogenic and nonodontogenic infections can be differentiated in the oral cavity. Odontogenic infections are more frequent and occur in the pulp, periapex or periodontium of the tooth. Neodontogenic infections occur with the penetration of microorganisms through the skin or mucous membrane.

Spreading of infection from the root canal - the endodontic way, or penetration of microorganisms through the periodont - the periodontic way, leads to infection of the tooth periapex. Infection is also possible *per continuitatem* with the neighbouring tooth.

Although in almost all investigations a small number of sterile samples is found of periapical tissue it is known that a periapical process cannot occur as a result of sterile pulpitis but is caused by bacterial infection through the apical foramen (9, 10). Penetration of bacteria through the apical foramen only occurs during the acute phase of inflammation.

Similar changes occur in periapical inflammation as in pulp infection. The inflammation can have a chronic or acute course, depending on the defensive strength of the organism and the number and virulence of the microorganisms. Mixed infections with a significant share of anaerobic bacteria are most frequent.

Earlier studies of the microbial flora of periapical lesions were performed by cultivating microorganisms from tissue samples which were obtained after tooth extraction. This was later changed to taking the microbiological sample directly from the periapical region during the operation - apicotomy.

Fungi are also isolated in periapical lesions. As in the case of endodontic infection *Candida* is most frequently found, although other fungi have been cultivated.

A case has been reported of protozoa from a periapical lesion (11).

In available literature the number of microbial species isolated in some microbiological samples from periapical lesions varies from 1 to 6, and the percentage of share of strictly anaerobes ranges from 30% to 80%. The most probable reason for the divergence in the investigations is the different methods used, transport and cultivation of samples. Another possible explanation is the fact that the microbial flora in odontogenic inflammation changes with the course of time in favour of anaerobic bacteria (12) and the microbiological finding depends on the phase of the inflammation in which the sample is taken.

The cellular wall of Gram positive bacteria is particularly important with regard to the changes which occur in the periapical area. The components of the cellular wall are responsible for the process leading to bone resorption and the occurrence of a cyst or granuloma (13).

Subjects and methods

Subjects

The investigation consisted of 60 patients from the Department of Oral Surgery Clinic of Maxillofacial Surgery in the Clinical Hospital "Dubrava", Zagreb.

The subjects were mainly patients from dental practices, sent to the Department with a diagnosis of a chronic ostitic process on the incisors, canines and premolars of the upper and lower jaw. The diagnosis was verified by a clinical examination and X--ray, and resection of the root apex was indicated.

The first group of subjects consisted of 40 patients (16 male subjects and 24 female) with a therapy resistant chronic ostitic process on one tooth. The mean age of the subjects was 33.1 years.

All subjects for whom apicotomy was planned had previously been subjected to preparation consisting of at least three treatments of mechanical dental treatment by their dentist involving the cleaning and widening of the root canal and rinsing with Na-hypochlor and physiological solution. After admittance to the Department, a final preoperative examination of the root canal was performed on the day of the operation for all subjects, with the object of eliminating any possible remaining infective material. At the same time final biomechanical instrumentation was performed with Kerr expanders and rinsing with 2.5% Na-hypochlor, with the obligatory use of rubber dam. The root canal was dried with a sterile paper stick and filled for apicotomy by the standard procedure - phosphate cement, and laterally well compressed gutta-percha through the apical foramen.

After the foregoing preoperative preparation the planned operation was performed. The course of the operation was normal, and material was taken from the inflammatory periapical area for microbiological analysis. The sample was placed in a transport medium and transported to the microbiological laboratory within two hours. After which the operation continued as usual.

The second group of subjects consisted of 20 patients with a periapical ostitic process on a tooth with a cemented metal reinforcement. This group comprised 14 females, mean age 42.3 years. For this group of subjects resection of the root apex with retrograde filling was performed instead of the usual preoperative preparation and canal filling. The approach to the periapical area and taking of the sample for microbiological analysis was identical to the first group of subjects, and the difference with regard to the procedure involved the method of retrograde filling of the root canal. Namely, all the teeth were retrogradely filled with amalgam.

No antibiotic therapy was taken by subjects from either group at least 7 days prior to taking the sample for microbiological examination.

Material for microbiological analysis

The following instruments were used for microbiological diagnosis: automatic thermostat, Mini-API, light microscope, room thermostat +35°C, refrigerator +4°C. The following were used for the microbiological diagnosis: swabs with transport medium (Venturi Transystem, Copan), API identification swabs for anaerobes, *Corynebacterium spp., Candida spp*. and anaerobic antibiogram, disposable plastic Petrij plates, glass Petrij plates and the following media: blood agar - Columbia Blood Agar, CDC Anaerobic Blood Agar, McConkey Agar. All the microbiological examinations were performed in the Department of Microbiology and Hospital Infections, Clinical Hospital Dubrava.

Statistical Analysis

Universal methods for statistical analysis were applied in order to test whether there were significant differences in distribution of the isolate between the two groups of subjects (patients or places of the sample taking). χ^2-test and Fisher's exact test were used to test the differences in the distribution of variables in the relatively small number of some variables.

McNemar's test was used to test the differences in distribution of dependent samples.

Universal analysis of variance (ANOVA) was used to test the differences in mean values. All the tests were performed with significance level of 0.05. The programme packet SAS system on Windows 95 platform was used in the statistical analysis.

Results

In the first group of subjects 40 microbiological samples were taken and in the second group 20. A total number of 103 isolates were isolated in the 60 samples (average 1.71 isolates per sample). Of the total number of isolates 19.4% were strictly anaerobic bacteria. A total number of 27 species of microorganisms were isolated, of which 26 were bacteria and one fungus - *Candida albicans*. Fourteen (51.8%) strictly anaerobic species were isolated.

Table 1 shows the microorganisms isolated in the periapex of subjects in the first group (open canal) and in the second group (closed canal) and the number of some isolates. Altogether 103 isolates were isolated, of which 101 were bacteria and two fungi - *Candida albicans*. In the first group 71 isolates were found - 70 bacteria and one fungi - *Candida albicans*. A total of 22 different bacteria were

isolated. In the second group 32 isolates were found - 31 bacteria and one fungi - *Candida*. A total of 9 different bacteria.

The table shows that some bacterial species occurred practically sporadically and they were thus excluded from further statistical analysis. Only those microorganisms which occurred at least three times in the total number of 60 samples were statistically analysed and mutually compared. The group included *Nonenterococcus species, Streptococcus species, Streptococcus viridans, Staphylococcus epidermidis, Veillonela species, Peptostreptococcus species* and *Enterococcus species*.

Table 2 presents a comparison of the occurrence of certain isolates in subjects in the first group (open canal) and the second group (closed canal). Statistically significant difference in occurrence was determined only for *Streptococcus spp*., in favour of the second group 875% - 42%).

Discussion

The number of microbial species in the oral cavity is today estimated to be from 300 to 500 (14, 15). Many of them have only recently been classified, and for many precise identification and classification have still to be done. All the microorganisms in the oral cavity are theoretically possible causative agents of infection. Opinions still differ on the role of viruses, fungi and protozoa (9, 11, 16-18) and numerous new investigations are concerned with their role in the aetiology of odontogenic inflammations.

Bacteria are undoubtedly the main causative agents of odontogenic infections. Some bacteria occur frequently in the results of investigations of microbial flora in odontogenic inflammation, while others never occur or only sporadically, as an incidental finding.

On the basis of available literature (19-26) it can be concluded that periapical lesions are rarely sterile and that the microorganisms isolated represent opportunists colonising through the root canal.

Compared to foreign literature our results are rather "meagre" with regard to the number of isolates and share of anaerobes.

Iwu (25) reported an average 3.4 isolates per sample and 45% strict anaerobes. In his investigation *Veillonela spp*. was also the most frequent anaerobe.

Virgil (27) found as many as 21 sterile findings. 50% of the isolates were anaerobes and *Peptostreptococcus micros* was the most frequent.

Brauner (21) determined as many as 6.8 bacteria per sample. The most frequently isolated were *Bifidobacterium, Streptococcus, Prevotella* and *Bacteroides*.

Samaranayake (23) found 22% sterile findings, 2.1 bacteria per sample and 20% strict anaerobes. The most frequent isolates were from the *Streptococcus viridans* group - 40%.

Wayman (28) reported 12% sterile samples, 2.3 isolates per sample, and 65% isolates were strictly anaerobes.

In his investigation Brook (26) found 17.9% sterile samples, two isolates per sample. 70.5% were strictly anaerobes, and the most frequent isolates were *Bacteroides and Streptococcus*.

Kobler (29) found 36.9% negative findings, 19 different bacterial species and 10.9% strictly anaerobes.

As can be seen from the above literature, there are no two completely identical investigations, and results differ from author to author. However, there is a mutual characteristic of microbial flora in which the results of our investigation agree. This in the first place refers to the polymicrobial composition and significant share of anaerobic causative agents.

Differences in the share of certain microbial species can be explained by geographic and time factors and the widespread use of antibiotics in the treatment of various infectious diseases (3, 6). The phase of inflammation in which the sample was taken has a great influence on the final outcome of the investigation, as also does the sampling method used, transport and cultivation, which must suit the anaerobic part of the microbial flora (14, 30, 31).

In our investigation 31 isolates belonging to 9 bacterial species + *Candida* were isolated from 20 closed periapical lesions. Only two isolates were strictly anaerobes - *Peptostreptococcus anaerobius and Lactobacillus acidophilus*. By far the most fre-

quent isolates were from the group of *Streptococci*, mainly *S. viridans*.

The small number of bacterial species corroborates the results of some foreign authors (21, 22, 25, 26, 28) and is understandable with regard to the nutritional and respiration conditions in the periapex of the tooth which facilitate less bacterial growth. In our investigation the share of strictly anaerobes in the total microbial flora of closed periapical lesions is surprising.

The aforementioned investigations of the microbial flora of closed periapical processes show data on the greater share of anaerobic bacteria in closed periapical processes. This is logical because of the anaerobic conditions prevailing in the periapex of the tooth with a closed root canal. In our investigation criteria for 'closed' was the existence of a cemented reinforcement. We did not take into consideration the existence of an eventual fistula, periradicular lesion or longitudinal fracture of the root canal, the existence of which can disrupt anaerobic conditions in the periapex. This may explain the small share of anaerobes in our investigation, i.e. disrupted anaerobic conditions. On the other hand we cannot explain the smaller share of anaerobes found in a closed periapical process, compared to an open periapical process, although we used an identical method of sampling, transport and cultivation for all teeth on which apicotomy was performed in our investigation.

Conclusion

1. The total number of isolates found in our investigation amounted to 103. The number of different species of microorganisms found was 27, of which more than half (14) were strictly anaerobes, and one fungi (*Candida*) which reflects the microbial flora in periapical infection.
2. The periapical inflammatory lesion in a tooth with an open root canal was caused by mixed polymicrobial infection, and the share of strict anaerobes was 25.3%. Bacteria from the streptococci group was most frequently isolated, and *Veillonela* and *Peptostreptococcus* anaerobes.
3. Analysis of microbial flora in closed periapical processes produced a somewhat narrower spectre of bacterial species. The share of anaerobes was surprisingly less than in open periapical lesions. Again bacteria from the streptococci group were most frequent.
4. The occurrence of *Streptococcus spp*. in the periapex of the tooth with an open root canal was statistically significantly more frequent.
5. The occurrence of *Candida albicans* in the periapex can be explained by contamination from the oral cavity, which is not considered to have any pathological effect on the development of odontogenic infection.