Analysis of Microbiological Results in Teeth with Chronic Apical Periodontitis

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Abstract: The invasion of the living microorganisms and their toxic products in endodontium and periapical structures is a significant problem in chronic apical periodontitis (CAP). This study analyzed the microbiological results of teeth with CAP. Microorganisms were isolated from the infected root canals of teeth with chronic apical periodontitis (primary or secondary) with or without the absence of clinical signs and symptoms. The microbiological diagnosis was made based on 79 teeth from 79 patients who fulfilled the inclusion criteria (n=79; 38 single-rooted and 41 multi-rooted teeth; 36 females and 43 males) with an average age of 40 years (age range = 18–62). The study investigated teeth with CAP with or without an endodontic treatment. The data was inputted and processed using statistical package SPSS 15.0.1. E. faecalis was the most frequently isolated microorganism (68.6%). The analysis of microbiological results in primary CAP (Group I) showed that there were no isolated microorganisms in 78.6% of the cases (22 cases). In contrast, in Group II, the first microbiological sample results were positive, with bacterial growth at 92.2% (47 cases). E. faecalis is a typical microflora in persistent CAPs performed with unsatisfactory endodontic treatment, and it is not isolated from teeth with primary CAP. E. faecalis is a resistant microorganism even after the application of chemomechanical preparation of the endodontic space.

Keywords: endodontics, enterococcus faecalis, microbiological analysis, periapical periodontitis, retreatment.

1. Introduction

The dynamic relationship between microorganisms with macroorganisms in infected radicular pulp tissue and periodontal ligaments is defined as local inflammation with typical signs of hard tissue resorption. This induces destruction of the periapical tissues and the formation of lesions in various stages of histopathological development. These chronic apical periodontitis (CAPs) are often referred to as periapical lesions [6,27]. The aim of contemporary endodontic retreatment is to ensure maximum successful regeneration of periapical structures by instrumentation of the root canal at the first appointment. This should be followed by the long-term intraconeal application of a calcium hydroxide dressing for one week. CAPs are the result of an inflammatory process in the periapical tissues initialized by nonspecific inflammatory and specific immunological responses to an infection in the endodontic space. Treatment is based on eliminating the microbes and their toxic products from the root canal, therefore removing the stimulus for either the cause or the persistence of CAP. The achieved degree of a maximum decontamination should be followed by an exact three-dimensional obturation of the endodontic space, which would prevent subsequent recontamination. Despite modern improvements in techniques, armamentarium, medicaments, and materials, a large percentage of CAPs are still the result of endodontic treatment that has already been performed. The frequency of CAP ranged from 2–10.5% in the study populations of several papers [1,3,16]. Some authors reported that CAP was found in 67.5% of the total number of studied devitalized teeth. He noted that the most common reasons for endodontic failure were the influence of microleakage and the unsatisfactory obturation of root canals [12, 30].

This present study aimed to analyze the microbiological results of teeth with CAP. The microorganisms were isolated from the infected root canals of teeth with chronic apical periodontitis (primary or secondary) with or without the presence of clinical signs and symptoms.

2. Material and Methods

The microbiological diagnosis was made on 79 teeth (n=79; 38 single-rooted and 41 multi-rooted) of 79 patients (36 females and 43 males) with an average age of 40 years (age range = 18–62) that met the inclusion criteria. The present study investigated teeth with chronic apical periodontitis (CAP) with or without an endodontic treatment. The criteria for inclusion of the patients in this clinical study were: lack of diagnosed common systemic diseases, lack of treatment with antibiotics in the last two months if endodontic treatment had been performed more than 12 months prior to the study. The clinical cases were grouped into two main groups. Group I consisted of teeth with chronic periapical lesions with primary endodontic treatment (n=51); Group II consisted of teeth with periapical lesions without endodontic treatment (n=28). All microbiological tests were performed at the same laboratory, /Cibalab, Sofia, Bulgaria/, in order to avoid discrepancies in results obtained from using different laboratories.

The clinical protocol included: removal of tartar and plaque, isolation of the operative field with a rubber dam, and disinfection of the operative field. After disclosure of the pulp chamber and/or removal of the sealer, the first sample was taken from the root canal with sterile paper points and was placed in a transport medium. The treatment protocol included a crown-down hybrid processing technique and an irrigation protocol with the application of the 5.25% NaOCl (activated with passive ultrasonic irrigation equipment), 17% EDTA, 40% citric acid, and 0.9% NaCl as the final rinse.

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In cases of endodontretreatment, Endosolv E (Septodont, France) and Endosolv R (Septodont, France) were used to remove the old canal filling. After instrumentation of the root canal system, access microbiological sample was taken using sterile paper points; it was then placed in a transport medium (VMMGA III gel). The endodontic access was sealed with a pellet in the pulp chamber with chlorhexidine (2%) and with a temporary filling. In 37 of the clinical cases, the endodontic treatment was performed over the course of twoappointments. In 42 of the clinical cases, the endodontic treatment was performed over the course of multiple appointments. This required the formation of two subgroups for each group: Subgroup A, the two-appointment treatment group, and Subgroup B, the multi-appointment treatment group.

3. Results

In 53 of the clinical cases of CAP, the first microbiological sample was positive (67.08%). Isolated microorganisms, mainly anaerobic microflora, confirmed the nature of the pathological changes in periapex-persistent chronic infection. Analysis of the microbiological results showed that the microorganisms were isolated in 78.6% of the primary CAP cases (n=22) (Group II).

Ricucci, et al.(2006) published similar results by analyzing 50 cases of primary CAP, and microorganisms were not detected in 32 (64%) of those cases [21]. If the microorganisms were not eliminated during preparation of the endodontic space, the dentin tubules were invaded and the microorganism reinfected the root canal. In contrast to the Group II results, the first microbiological sample in Group I was positive for bacterial growth (92.2% [47 cases]). E. faecalis is the most common microorganism isolated from the root canal in secondary CAP (Group I; 68.6%). This high percentage demonstrates the microorganism’s ability to penetrate into the dentin tubules, to adhere strongly to collagen, and to resist the irrigation solutions used in the endodontic treatment protocol. The study results are evaluated and tabulated in Table 1 and Table 2.

### Table 1: Comparison of percentage shares of Group I and II after the first microbiological sample

<table>
<thead>
<tr>
<th>Isolated microorganisms</th>
<th>Code</th>
<th>Descriptive statistics (Group I)</th>
<th>Descriptive statistics (Group II)</th>
<th>Comparison of percentage Shares into two groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n=51</td>
<td>n=28</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1</td>
<td>68.6% (35 cases)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>2</td>
<td>50.9% (26 cases)</td>
<td>17.8% (5 cases)</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3</td>
<td>37.3% (19 cases)</td>
<td>10.7% (3 cases)</td>
<td>P = 0.360</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>4</td>
<td>21.6% (11 cases)</td>
<td>7.1% (2 cases)</td>
<td>P = 0.029</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>5</td>
<td>31.4% (16 cases)</td>
<td>21.4% (6 cases)</td>
<td>P = 0.910</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>6</td>
<td>19.6% (10 cases)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Protens Mirabili</td>
<td>7</td>
<td>3.92% (2 cases)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td>8</td>
<td>3.92% (2 cases)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Negative samples</td>
<td></td>
<td>7.8% (4 cases)</td>
<td>78.6% (22 cases)</td>
<td>P = 0.000</td>
</tr>
</tbody>
</table>

n = number of teeth

### Table 2: Comparison of percentage shares of Group I and II after the second microbiological sample.

<table>
<thead>
<tr>
<th>Isolated microorganisms</th>
<th>Code</th>
<th>Descriptive statistics (Group I)</th>
<th>Descriptive statistics (Group II)</th>
<th>Comparison of percentage Shares into two groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n=51</td>
<td>n=28</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1</td>
<td>17.64% (9)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>2</td>
<td>5.8% (3)</td>
<td>1.96% (1)</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Pseudomonas Aeruginosa</td>
<td>3</td>
<td>3.92% (2)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>4</td>
<td>1.96% (1)</td>
<td>1.96% (1)</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>5</td>
<td>3.92% (2)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>6</td>
<td>0.00% (0)</td>
<td>3.20% (1)</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Protens Mirabili</td>
<td>7</td>
<td>0.00% (0)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td>8</td>
<td>1.96% (1)</td>
<td>-</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Positive samples</td>
<td></td>
<td>35.29% (18 cases)</td>
<td>14.28% (4 cases)</td>
<td>P = 0.000</td>
</tr>
</tbody>
</table>

n = number of teeth; A = Subgroup A; B = Subgroup B.
A – Group I; 1–8 – codes of microorganisms: 1 – E. Faecalis; 2 – Str. species; 3 – Pseudomonas aeruginosa; 4 – Prevotella intermedia; 5 – C. albicans; 6 – Porphyromonas gingivalis; 7 – Proteus Mirabili; 8 – Peptostreptococcus micros; X – statistically significant difference.

Figure 1: Distribution of microorganisms in both groups studied.

The samples with isolated microorganisms in Group I (35.29%; 18 cases) and Group II (14.28%; 4 cases) formed the basis for the creation of two subgroups for each of the two study groups. The samples with isolated microorganisms in Group I (35.29%; 18 cases) and Group II (14.28%; 4 cases) formed the basis for the creation of two subgroups for each of the two study groups. This was the case for the two major groups of positive results. A multi-appointment treatment was performed for optimal decontamination. The results are presented in Figure 1. In Group I, no statistically significant differences in percentages were found for the various isolated microorganisms, coded "X" (Table 3), using the chi-square test. Statistical processing of the results showed that the isolation of Proteus Mirabili (3.92%) and Peptostreptococccus micros (3.92%) could not be applied to microorganisms typically presented in persistent CAP with unsatisfactory endodontic treatment (p = 0.491). The resistance of E. faecalis at a higher pH (capable of development at a pH of 9.6 and tolerant even at a pH of 12.1) suggested that a highly alkaline environment increases the microorganism's ability to adhere to type I collagen.

4. Discussion

Over a century ago, Miller (1890) proved that several different types of bacteria are present in necrotic pulp tissue. Half a century later, Kakehashi, Stanley and Fitzgerlad(1965) proved that they do not develop in the sterile pulp chambers of rabbits in an oral environment [14]. These rabbits were compared with the rabbits in the control group that were exposed to conventional oral microflora and sterile pulp chambers of rabbits in an oral environment [14]. Half a century later, Kakehashi, Stanley and Fitzgerlad(1965) proved that they do not develop in the sterile pulp chambers of rabbits in an oral environment [14]. These rabbits were compared with the rabbits in the control group that were exposed to conventional oral microflora and sterile pulp chambers of rabbits in an oral environment [14]. These rabbits were compared with the rabbits in the control group that were exposed to conventional oral microflora and sterile pulp chambers of rabbits in an oral environment [14]. 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These rabbits were compared with the rabbits in the control group that were exposed to convention...
samples from another researcher[9]. The proven ability of *E. faecalis* to penetrate into dentinal tubules [18,28], strongly adhere to collagen [17], and resist the irritation solutions used in the endodontic treatment protocol, were confirmed by the results obtained in this work. After chemomechanical preparation, the percentage of isolated *E. faecalis* was 17.64%. Gomes et al.(2003) determined that, even after treatment with NaOCl (5.25%) and Ca(OH)2 (pH = 12), *E. faecalis* is a microorganism with the highest resistance to chemomechanical preparation of the root canal system [7].

In addition, we speculate that in spite the fact that scientific papers have observed a low success rate for endodontic retreatment, the prevailing data shows that the success rate is higher than the rate of teeth treatments with negative samples relative to *E. faecalis*. This concurs with the results of the present study.

5. Conclusion

*E. faecalis* was the most frequently isolated microorganism in this present study (68.6%). It was isolated from teeth with CAP and teeth with unsatisfactory endodontic treatment. *E. faecalis* is a typical microflora in persistent CAPs performed with unsatisfactory endodontic treatment, and it was not isolated from teeth with primary CAP. *E. faecalis* is a resistant microorganism even after chemo-mechanical preparation of the endodontic space. The analysis of microbiological results for primary CAP (Group II) shows that, in 78.6% of the cases (n=22), no microorganisms were isolated. In contrast to the results from Group II, the first microbiological sample in Group I showed positive bacterial growth in 92.2% of the cases (n=47).

6. Acknowledgments

The authors deny any conflicts of interest related to this study.

References


[23] Sheely E, Roberts G. Use of calcium hydroxide for apical barrier formation and healing in non-vital