BACTERIAL REDUCTION IN INFECTED ROOT CANALS TREATED WITH CALCIUM HYDROXIDE USING HAND AND ROTARY INSTRUMENT: AN IN-VIVO STUDY

ABSTRACT

Background and Aim: The aim of this in vivo study was to evaluate the microbial flora in infected root canals after the treatment with and without calcium hydroxide (Ca(OH)₂) dressing using hand and rotary instruments.

Subjects and Methods: Twenty-eight patients were selected. After access cavity preparation completed, the first culture were taken from each root canal with sterile paper points. Four groups were formed. Group I and II were instrumented with ProTaper instruments, group III and IV were instrumented step-back technique with K files. Group I and III were medicated with Ca (OH)₂ paste. One week after, the second culture was taken and the canals were filled. All samples were submitted to microbiological processing to evaluate anaerobe and aerobe microorganism. The results statistically analyzed with McNemar and Chi-Square tests at p<0.05.

Results: There was a significant decrease in bacteria elimination in Group III compared to the other groups (p<0.05). Groups, in which Ca(OH)₂ was applied, were found significantly better to eliminate bacteria in root canals than the groups without Ca(OH)₂.

Conclusion: Better root canal disinfection could be obtained by mechanical preparation with application of Ca(OH)₂ for one week.

Key words: Calcium Hydroxide, Microbiology, Root Canal Treatment, Rotary Instruments

Submitted for Publication: 10.05.2011
Accepted for Publication: 06.21.2012
It is well known that remaining bacteria, either in the root canal space or in parts of root canal system that are not filled, is the main cause for root canal treatment failure. Thus, removal of vital and necrotic pulp tissue, microorganisms and their toxins is an important part of endodontic treatment. Unfortunately, studies have shown that both stainless steel file systems which have been used for years and Ni-Ti rotary instruments that have gained popularity recently have lead to inadequate disinfection of root canal space because of the complexity of root canal morphology, apical ramifications, isthmuses, other morphological challenges and bio-film form of root canal bacteria. As a result of technological developments, Ni-Ti rotary instruments have gained increased taper and different designs that can significantly improve the cleaning and shaping procedure. Many studies have been conducted to compare Ni-Ti rotary instruments with stainless steel instruments. However, there have been few in-vivo studies in which Ni-Ti rotary instruments provided better results in reducing or eliminating intra-canal bacteria, compared with stainless steel instruments. During endodontic treatment, both mechanical preparation and intra-canal dressing are used to reduce or eliminate bacteria. Some studies have shown that mechanical preparation significantly decreases bacteria in the root canal. Yet, in approximately 20%-50% of the canals, remaining bacteria was observed. For this reason, in addition to mechanical preparation, different concentrations and types of irrigation solutions and various medicaments are used to remove microorganisms and to achieve disinfection. It has been reported that the use of sodium hypochlorite (NaOCl) in different concentrations during mechanical preparation decreases the number of bacteria. Despite the use of this solution, however, positive culture was obtained in 40-60% of the root canals. In addition to irrigation solutions, intra-canal medicaments are used to remove bacteria and to disinfect the root canal systems. Calcium hydroxide (Ca(OH)_2) has been the most frequently used intra-canal medicament due to its antimicrobial effect, osteoclastic activity and suitable tissue response. Whether or not there is an apical lesion, in cases with necrotic pulp, multiple-visit treatment and the use of Ca(OH)_2, as medicament is recommended for a week to achieve the desired disinfection.

This in-vivo study takes stock of results obtained in previous research highlighted above and aims to assess the amount of microbial flora in infected root canals by using Ni-Ti rotary instruments and stainless steel instruments, once with and once without the use of Ca(OH)_2.

**Subjects and Methods**

**Case selection**

Patient population consists of 28 people (18 female, 10 male) who frequented Hacettepe University, Faculty of Dentistry, Department of Endodontics. In total, 28 teeth (9 maxillary incisors, 2 maxillary premolars, 6 maxillary molars, 2 mandibular incisors, 1 mandibular premolar and 8 mandibular molar) have been treated. Patient selection criteria were as follows:

1. The teeth with intact pulp chamber walls,
2. Necrotic pulps, negative response to the pulp tests (electrical and cold),
3. Clinical and radiographic evidence of chronic apical periodontitis lesions,
4. Teeth from patients who have not received antibiotic therapy within the preceding month,
5. Patients without swelling or any symptoms or periodontal problems.

Before initiation, the study was approved by the Ethics Committee of the Hacettepe University and informed consent was obtained from each patient.

**Endodontic Treatment and Sampling Procedures**

After the clinical and radiographic examination of the patients, each tooth was cleaned of plaque, calculus and attachments. All decay and unsupported tooth structure were removed and canal orifices were localized after access cavity preparation. Following the rubber-dam isolation, all tooth surfaces were cleaned with a cotton pellet immersed in 2.6% NaOCl. A #15 K file was inserted into canals for apical patency; then, the first root canal sample was taken. The sterile paper point was placed in each root canal to a level approximately 1 mm short of the apex, based on diagnostic radiographs, and was used to soak up the fluid in the canal. Each paper point was kept in the canal for 3 minutes. Paper points were then placed into aseptically tubes containing 1 mL tiyoglukonated buyyon.

Four groups were formed, each with 7 teeth. After estimating the working length, root canals in Groups I and II were prepared with ProTaper Ni-Ti rotary instruments (Dentsply-Maillefer, Ballaigues, Switzerland) to a master apical size of #30 using an endodontic micromotor (X-Smart, Dentsply, ...
InFECTED RooT CAnALs TREATED wITH CAlCIUM HyDRoxIDE

Maillefer). RC-Prep (RC-Prep; Premier Dental, Norristown, PA) chelating agent was used for lubrication. In Groups III and IV, root canals were prepared with the step-back technique using stainless steel files, with the master apical file being determined according to each tooth’s anatomy. The canals were irrigated with 0.5 ml 2.5% NaOCl after each file. In all cases, chemo-mechanical preparation was completed at the first appointment. After the instrumentation, 5 ml 2.5% NaOCl was applied as final flush; then each canal was dried up by using sterile paper point (Spident, Incheon, Korea). In Groups I and III, root canals were filled with Ca(OH)$_2$ (SURE-Paste, Korea). Ca(OH)$_2$ paste was placed by using a lentulo spiral and packed with a cotton pellet at the level of canal orifice. A radiograph was taken to ensure proper placement of the paste in the canal. Then the access cavities were filled with at least 4 mm of a temporary filling material (Nucavfil; PSP Dental, Belvedere, Kent, UK).

The second appointment was scheduled for the following week. At this time, the tooth was isolated with rubber-dam and the operative field was disinfected. In Groups I and III where Ca(OH)$_2$ was applied, Ca(OH)$_2$ paste was rinsed out of the canal by using 2 ml sterile saline solution and master apical files. In Groups II and IV, after temporary filling material was removed, each canal was irrigated with only 2 ml sterile saline solution. The second culture was taken from each canal as outlined above. Subsequently, the canals were filled with gutta-percha (Diadent, Seoul, Korea) and AH-26 root canal sealer (Dentsply Detrey, Konstanz, Germany) using cold lateral compaction technique. The tooth was temporized with glass ionomer cement and a permanent restoration was planned.

**Microbiologic Analysis**

Root canal samples were taken with sterile paper points which were left in the canal for at 3 minutes and placed in 1 ml of thioglycolate broth. Each sample was vortexed and 10μl of the sample was transferred onto each of the following media: Sheep blood agar, chocolate agar, MacConkey agar and Schaedler agar. All media were incubated overnight in 5-10% CO$_2$ at 37 °C (-/+ 2), except Schaedler agar which was incubated in anaerobic atmosphere for 48 hours. Colony counts were determined for each group of bacteria with different colony morphologies and subcultured for identification. Aerobic bacteria were identified to genus level by Gram stain morphology, catalase and coagulase tests, growth on bile-esculin agar, growth in 6.5% NaCl and CAMP reaction. Anaerobic bacteria were identified to species level by catalase test, indole reaction and Crystal System (Becton Dickinson, USA).

**Statistical Analysis**

A non-parametric test (McNemar) was used for determining the differences between first and second measurement in the intergroup and the Chi-Square test was used for evaluating the differences between the groups. A p value < 0.05 considered as statistically significant.

**RESULTS**

Table 1 shows the total distribution of bacterial types at the beginning of access cavity preparation in the first visit and at the end of preparation in the second visit. While the total number Colony Forming Unit (CFU) of the first samples was 551170 CFU/ml, after instrumentation, irrigation and applying Ca(OH)$_2$ the CFU count had dropped significantly to total 391300 CFU/ml. In culture results, 44% of the microbial flora of root canals was found to be aerobic while 46% of the flora was anaerobic. Most observed aerobic bacteria species was Alpha hemolitic streptococcus with a ratio of 28.8%. Most observed anaerobic bacteria species was Veillonella spp with a ratio of 22.8%.

In all experiment groups, when the final measurements of difference in the decrease of both aerobic and anaerobic bacteria were evaluated (Table 2). Statistically significant difference was only seen in Group III in which Ca(OH)$_2$ was applied after cleaning and shaping procedures with hand instrument (p<0.05). There is a significant decrease in bacteria elimination in Group III compared to other groups. When initial and final changes in cultures were evaluated, Group IV registered the highest level of bacteria compared to other groups in which cleaning and shaping procedures were carried out with hand instrument without using Ca(OH)$_2$ (p=0.0036). Groups in which Ca(OH)$_2$ was applied proved to be significantly better in eliminating bacteria in root canals compared to groups in which Ca(OH)$_2$ was not applied (Figure 1). Cleaning and shaping procedures with hand instrument were significantly better than rotary instrument groups in eliminating bacteria from root canals (p=0.03) (Figure 2).

**DISCUSSION**

An effective endodontic treatment requires the eradication of bacteria or at least the reduction in bacterial counts in the root canal space.\(^1\) Chemo-mechanical preparation of root canal using different types and concentrations of irrigation
Table 1. The total distribution of bacterial types in the first visit and second visit

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>CFU</th>
<th>Prevalance</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobe Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfa Hem. Strep.</td>
<td>34.5</td>
<td>253500</td>
<td></td>
<td>28.81664</td>
<td></td>
</tr>
<tr>
<td>Coagulase-Stap</td>
<td>29.14</td>
<td>420800</td>
<td></td>
<td>47.83449</td>
<td></td>
</tr>
<tr>
<td>Neisseria</td>
<td>14.54</td>
<td>10400</td>
<td></td>
<td>1.182221</td>
<td></td>
</tr>
<tr>
<td>S. Viridans</td>
<td>5.46</td>
<td>17100</td>
<td></td>
<td>1.943844</td>
<td></td>
</tr>
<tr>
<td>Difteroid</td>
<td>5.46</td>
<td>24100</td>
<td></td>
<td>2.73957</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10.9</td>
<td>153800</td>
<td></td>
<td>17.48323</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>879700</td>
<td></td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td><strong>Anaerobe Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veillonella ssp</td>
<td>34.70</td>
<td>16870</td>
<td></td>
<td>22.86837</td>
<td></td>
</tr>
<tr>
<td>Actinomyces</td>
<td>8.69</td>
<td>6000</td>
<td></td>
<td>8.133888</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>13.17</td>
<td>8200</td>
<td></td>
<td>11.11563</td>
<td></td>
</tr>
<tr>
<td>Eurobacterium lim</td>
<td>4.34</td>
<td>10000</td>
<td></td>
<td>13.55565</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>8.69</td>
<td>11500</td>
<td></td>
<td>15.58999</td>
<td></td>
</tr>
<tr>
<td>Bacteriodes cap</td>
<td>4.34</td>
<td>5000</td>
<td></td>
<td>6.777823</td>
<td></td>
</tr>
<tr>
<td>Prevotella</td>
<td>4.34</td>
<td>5300</td>
<td></td>
<td>7.184492</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>21.73</td>
<td>10900</td>
<td></td>
<td>14.77565</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>73770</td>
<td></td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

CFU: Colony Forming Unit

Table 2. Result of one week treatment according to groups

<table>
<thead>
<tr>
<th></th>
<th>Rotary with Ca(OH)_2</th>
<th>Rotary w/o Ca(OH)_2</th>
<th>Manual with Ca(OH)_2</th>
<th>Manual w/o Ca(OH)_2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Elimination</td>
<td>7</td>
<td>53.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Repeated-growth</td>
<td>2</td>
<td>15.4</td>
<td>5</td>
<td>41.7</td>
</tr>
<tr>
<td>Stagnation</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>Reduction</td>
<td>2</td>
<td>15.4</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>New</td>
<td>2</td>
<td>15.4</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>100</td>
<td>13</td>
<td>100</td>
</tr>
</tbody>
</table>

n: The number of species of microorganism

Solution has been shown to be critical step for reduction of bacterial populations in the root canal but about 40% to 60% of the canals still yield positive cultures after instrumentation and irrigation.9,15 Bacteria in the infected root canal are mostly anaerobic bacteria.16 Our study shows that 44% of the microbial flora of root canals was aerobe while 46% was anaerobe. According to the results reported by Sundquist et al.17 Byström & Sundqvist18 Baumgartner & Falk,19 Siqueira & Lopes,20 and Sassone et al.21, the most microbial flora in infected root canals is anaerobic bacteria. These authors concluded that the infection in infected root canal is mainly polymicrobial, with anaerobic flora being less
frequent. The results reached in our study conflict with those reported by the mentioned authors. This may be explained by the method we applied for the identification of microbes in root canal.

Although cleaning, shaping and irrigating the canal greatly reduce the number of bacteria, it is generally believed that the number of bacteria can also be controlled by placing an inter-appointment dressing such as Ca(OH)$_2$ within the prepared root canal. Estrela et al. analyzed the antimicrobial properties of Ca(OH)$_2$ and concluded that the latter affects the cell membrane of both aerobe and anaerobe bacteria. Sjögren et al. clinically evaluated the antibacterial effect of Ca(OH)$_2$ as a short-term intra-canal dressing by applying the medicament for 7 days in root canals of teeth with periapical lesions. These authors showed that the 7 day dressing effectively eliminated bacteria which survived biomechanical instrumentation of the canal. The present study also shows that groups in which Ca(OH)$_2$ was applied were significantly better in eliminating bacteria in root canals compared with groups in which Ca(OH)$_2$ was not applied. After the final measurement, a statistically significant difference was observed only in Group III (treated with Ca(OH)$_2$ and hand instruments). Our results parallel those of Sakamoto et al. showing the success of hand instrumentation and treatment with Ca(OH)$_2$ in 97% of cases. De Rossi et al. investigated the effect of Ca(OH)$_2$ in infected root canals using hand and rotary instruments and showed that, regardless of the instrumentation technique, the use of intra-canal dressing is important in the endodontic treatment for the elimination of bacteria in the root canal. The present study demonstrates that instrumentation, irrigation and applying Ca(OH)$_2$ significantly reduces the total number of microorganisms, namely approximately to 16 % of the original number. Peters et al. found that the total number of microorganisms was reduced by 0.18 % after instrumentation, irrigation and dressing with Ca(OH)$_2$ on infection in pulpless teeth with periapical lesions. They also concluded that such reduction was not related to the use of Ca(OH)$_2$ for 4 weeks. The difference in our findings might relate to the duration of the use of Ca(OH)$_2$ and the transport media used. In our study, Ca(OH)$_2$ was applied for one week as opposed to four weeks.

In this study, we analyzed the effectiveness of hand and rotary instrumentation in the elimination of bacteria from root canal. Our results show that hand instruments are far better than rotary instruments in cleaning and shaping \((p=0.03)\). Even if the crown-down technique was the basis for the preparation with rotary files, taper and apical diameter caused changes in the preparation. Similarly, in the preparation with hand files, the taper and apical diameter was different. In all Groups, master apical file was determined according to each tooth’s number and anatomy. This might have had an impact on dentin elimination and bacterial reduction. ProTaper instruments have active cutting surface which allows such instruments to progress easily in the root canal and remove more infected dentin from the root canal compared with hand instruments. However, although rotary instruments appear to be better than hand instruments in reducing bacteria counts, in this study, the Groups prepared with hand files turned out to be better than rotary files on bacterial reduction. The results of this study conflict with those of Chuste-Guillot et al., Limongi et al. and Beun et al. who concluded that hand and rotary instruments were not significantly different in eliminating bacterial flora from root canal. Evans et al. also showed no difference between the hand-file step-back and NiTi Quantec systems regarding pulp and pre-dentin elimination.
CONCLUSION

Our findings show that regardless of the technique and the instrument, a similar quantity of bacterial reduction may be achieved. A good root canal disinfection may be obtained by mechanical preparation coupled with the application of Ca(OH)_2 for one week.

REFERENCES


