Quantitative Assessment of apically Extruded Bacteria using different Instrumentation Techniques and Preparation Taper

1Harsh Priyank, 2Vinisha Pandey, 3Achla Sethi, 4Vinay J Sharma, 5Harleen Bali, 6Ramandeep S Punia

ABSTRACT

Background: Cleaning and shaping of the pulp canal is one of the most important steps of endodontic therapy. Serious complications occur by the apical extrusion of bacteria during the instrumentation procedures. Both crown-down (CD) and full-length linear motion (FM) techniques are routinely used as a component of taper rotary instrument procedures for achievement of thorough cleaning and shaping of the pulp canal space. Hence, we aimed for this study to assess the change in the amount of apically extruded bacteria using CD and FM instrumentation techniques produced by differences in taper between the instruments used during biomechanical preparation of root canals.

Materials and methods: The present study included assessments of 132 extracted maxillary central incisor teeth. To achieve a uniform teeth length of 21 mm, the height of the tooth crown was reduced for preserving the coronal portion of teeth. A modified glass vial model was constructed for the estimation of amount of bacterial extrusion through the apical region. For filling of each pulp canal specimen, 20 mL of Enterococcus faecalis suspension was used followed by the use of a number 10 K-file for carrying the bacteria down the lengths of pulp canals. All the contaminated teeth specimens were divided into six study groups with groups I to III containing specimens prepared in the CD manner, while groups IV to VI contained specimens prepared in the FM manner. Six teeth were taken as negative control with three specimens with each technique, and another six specimens were taken as positive controls. Cultivable bacterial counts were determined by evaluating 100 mL saline solution from each vial followed by its inoculation on blood agar. All the colony-forming unit (CFU) values were log-transformed (base 10), and the results were analyzed by Statistical Package for the Social Sciences software.

Results: A significantly lower quantity of CFU values was observed during CD instrumentation procedures with 0.02 files in comparison with all other study groups. However, while comparing both the instrumentation procedures when different taper files, other than 0.02 taper, were used for biomechanical preparation of root canal, nonsignificant results were obtained.

Conclusion: With 0.02 taper preparations, significantly less amount of extrusion of bacteria is associated when done with CD technique.

Clinical significance: No change in the amount of apical extrusion of bacteria will be seen by changing the type of instrumentation procedures. Amount of bacteria extruded can be minimized using 0.02 taper.

Key words: Bacteria, Instrumentation, Taper.


Source of Support: Nil
Conflict of Interest: None

INTRODUCTION

One of the most important component steps for the successful completion of root canal therapy is the complete and thorough cleaning and shaping of root canal space for the purpose of removal of inflamed and necrotic pulpal tissue.1 During the process of root canal instrumentation,
the following components can be extruded beyond the apical foramen:

- **Dentinal chips**
- **Root canal tissue fragments**
- **Necrotizing and inflamed tissue**
- **Intracanal medicaments**
- **Microorganisms**

This can impose a serious problem as the apically extruded necrotic materials and microorganisms can cause posttreatment pain or a flare-up. Although these risk factors are significantly decreased by an accurate control of the working length, periapical extrusion of these components can result in serious posttreatment complications, such as flare-ups, pain, and swelling, which can further result in extreme discomfort to the patients resulting in inter-appointment emergency. In the present treatment scenario, apical extrusion of debris is associated with all types of preparation techniques and instrumentation procedures. This occurs even with clinicians prepare the root canal short of the apical terminus.

Both crown-down (CD) and full-length linear motion (FM) techniques are routinely used as a component of taper rotary instrument procedures for the achievement of thorough cleaning and shaping of the pulp canal space. Literature quotes that significantly less bacterial extrusion is associated with CD preparation techniques in comparison with FM instrumentation. There is paucity of studies in the literature that have assessed the impact of pulp canal taper on the apical extrusion of necrotic debris and bacteria. Hence, we aimed for this study to assess the change in the amount of apically extruded bacteria using CD and FM instrumentation techniques produced by the difference in taper between the instruments used during biomechanical preparation of root canals.

**MATERIALS AND METHODS**

This study was conducted in the Department of Conservative Dentistry and Endodontics of the dental institute, and included the assessment of 132 extracted maxillary central incisor teeth. All the teeth, after extraction, were immediately placed in normal saline solution for storage. Inclusion criteria for the present study included:

- Single-rooted maxillary central incisors
- Teeth with straight pulp canals
- Teeth with initial apical diameter approximate to International Organization for Standardization size #15 K-file

All the extracted teeth were cleaned carefully for removing any remnants of bone, calculus, and necrotic or physiologic soft tissue. Periodontal curettes were used for removing these fragments. To achieve a uniform teeth length of 21 mm, the height of the tooth crown was reduced for preserving the coronal portion of teeth. The crown portion was preserved because it acts as a reservoir for irrigation solution. Expiration of the root canal remnants was done with the help of a barbed broach, and 1 mm short of apex (apical foramen), the working length was evaluated. Cyanoacrylate glue was used for sealing the root ends of the entire teeth specimen once they were dried. This was done for creation of a reservoir in root canals for contamination with a suspension of *Enterococcus faecalis*. Construction of a modified glass vial model was done for the estimation of amount of bacterial extrusion through the apical region. A hole was created through the center with the purpose of holding the tooth specimen in an upright position. Insertion of the tooth specimens into the rubber stoppers under pressure was performed, and glue was used for fixing the samples to the stoppers through their cementoenamel junction. For the prevention of bacterial microleakage, the vials were coated on their outer surfaces by two to three coats of nail varnish. The entire system was then sterilized for obtaining a contamination-free environment. For equalization of the pressure inside and outside the vial, a sterile 27-gauge needle was inserted alongside the rubber stopper. From fresh 24-hour bacteria grown in brain–heart infusion broth, a standard suspension of *E. faecalis* was formulated. For ensuring the bacterial concentration of 1.5 × 10^8 colony-forming units (CFUs) mL⁻¹, adjustment of the turbidity of the suspension was done. About 20 mL of *E. faecalis* suspension was used for filling of each pulp canal specimen, followed by use of a number 10 K-file for carrying the bacteria down the lengths of pulp canals. This was followed by incubation of the infected pulp canal specimens at 37°C for 24 hours. Filing of the vials was done with 10 mL of saline solutions before the commencement of the instrumentation procedure of root canals. For the creation of a standard apical patency, placement of a sterile number 15 K-file was done 1 mm beyond the apical foramen. All the contaminated teeth specimens were divided into six study groups as shown in Table 1, with 20 specimens in each group. In groups I to III, preparation of the canals was done in the CD manner, while in the groups IV to VI, preparation of the canals was done in the FM manner. Six teeth were taken as negative control (noninfected) with three specimens with each technique, 10 K-file for carrying the bacteria down the lengths of pulp canals. This was followed by incubation of the infected pulp canal specimens at 37°C for 24 hours. Filing of the vials was done with 10 mL of saline solutions before the commencement of the instrumentation procedure of root canals. For the creation of a standard apical patency, placement of a sterile number 15 K-file was done 1 mm beyond the apical foramen. All the contaminated teeth specimens were divided into six study groups as shown in Table 1, with 20 specimens in each group. In groups I to III, preparation of the canals was done in the CD manner, while in the groups IV to VI, preparation of the canals was done in the FM manner. Six teeth were taken as negative control (noninfected) with three specimens with each technique.

**Table 1: Distribution of samples into various study groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Technique</th>
<th>Taper</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CD</td>
<td>0.02</td>
</tr>
<tr>
<td>II</td>
<td>CD</td>
<td>0.04</td>
</tr>
<tr>
<td>III</td>
<td>CD</td>
<td>0.06</td>
</tr>
<tr>
<td>IV</td>
<td>FM</td>
<td>0.02</td>
</tr>
<tr>
<td>V</td>
<td>FM</td>
<td>0.04</td>
</tr>
<tr>
<td>VI</td>
<td>FM</td>
<td>0.06</td>
</tr>
</tbody>
</table>
and another six specimens were taken as positive controls (previously infected). After the completion of pulp-canal preparation procedure, cultivable bacterial counts were determined by evaluating 100 mL saline solution from each vial followed by its inoculation on blood agar with the help of the quantitative inoculation technique. Following incubation at 37°C for 24 hours, calculation of the CFUs for each tooth specimen was done. For statistical analysis, all the CFU values were log-transformed (base 10), and the results were analyzed by Statistical Package for the Social Sciences software version 16.0. Student’s t-test and Tukey’s honest significant difference test were used for the assessment of the level of significance. The p < 0.05 was taken as significant.

RESULTS

Distribution of all the specimens in various groups is shown in Table 1. Table 2 highlights the mean values and standard deviation in all the experimental groups. No growth was observed in the negative control group, while a positive bacterial growth was associated in all the positive control groups during assessment after experimental time interval. A significantly lower quantity of CFU values was observed during CD instrumentation procedures with 0.02 files in comparison with all other study groups (p < 0.05) (Table 2 and Graph 1). However, no statistically significant results were obtained while comparing both the instrumentation procedures when different taper files, other than 0.02 taper, used for biomechanical preparation of root canal (p > 0.05).

DISCUSSION

In the presence of dense inflammatory cell infiltrate and intense immune response, pain and clinical swellings usually accompany the extrusion of cellular debris and microorganisms in the apical areas during the process of cleaning and shaping the root canals. The mechanical cleaning process of the pulp canals is often associated with this undesired consequence, and none of the techniques of cleaning and shaping of root canals avoids apical debris extrusion. Therefore, routine investigation is carried out in search of a technique that avoids such negative consequences. Variation has been seen in the amount of debris extruded apically, which further depends on the kinematics, amount of root canal filing done, taper, cross-section, and efficacy of the files used for preparing the root canal. Hence, we aimed for the present study to assess the change in the amount of apically extruded bacteria using CD and FM instrumentation techniques produced by differences in taper between the instruments used during biomechanical preparation of root canals.

In this study, we used three different types of taper systems, i.e., 0.02, 0.04, and 0.06 (Table 1). Apical extrusion of all the bacteria resulted from all the instrumentation types and procedures. With the exceptions of results obtained with 0.02 taper preparations, no significant results were obtained while comparing the amount of bacterial extrusion in different types of taper and with

Table 2: Correlation of distribution of apically extruded E. faecalis (CFU mL⁻¹)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CD-02 2.251 ± 0.16</td>
<td>1.25</td>
<td>3.15</td>
</tr>
<tr>
<td>II</td>
<td>CD-04 2.812 ± 0.12</td>
<td>2.18</td>
<td>3.46</td>
</tr>
<tr>
<td>III</td>
<td>CD-06 2.842 ± 0.09</td>
<td>2.22</td>
<td>3.39</td>
</tr>
<tr>
<td>IV</td>
<td>FM-02 2.872 ± 0.12</td>
<td>2.25</td>
<td>3.24</td>
</tr>
<tr>
<td>V</td>
<td>FM-04 2.932 ± 0.50</td>
<td>1.92</td>
<td>3.64</td>
</tr>
<tr>
<td>VI</td>
<td>FM-06 2.992 ± 0.11</td>
<td>2.39</td>
<td>3.34</td>
</tr>
</tbody>
</table>

Difference in superscripts shows statistically significant difference among various sample groups (p<0.05)

Graph 1: Descriptive values of distribution of apically extruded E. faecalis (CFU mL⁻¹)

The Journal of Contemporary Dental Practice, September 2017;18(9):1-4
CD and FM instrumentation approaches (Table 2). Our results were in correlation with the results obtained by Aksel et al., who observed similar findings in their study. Nevares et al. evaluated and compared the amount of debris extruded periapically in canals prepared by various systems used in reciprocating and continuous motions. They analyzed 60 mandibular teeth, and divided them randomly into three study groups with 20 specimens in each group. These groups were the Reciproc (REC) group, WaveOne (WO) group, and HyFlex CM (HYF) group respectively. On an analytical balance, one Eppendorf tube per tooth was examined and weighed. As per manufacturer’s instructions, pulp canals were instrumented. To a total of 9 mL, 2.5% sodium hypochlorite solution was used for standard irrigation. Incubation of the teeth specimens after removal from the Eppendorf tubes was done at 37°C for 15 days for evaporating the liquid. They again weighed the tubes and noted and calculated the difference between the initial and final weights. They observed that apical extrusion of debris resulted from all the three types of systems. However, significantly more debris was extruded when prepared with 0.02 taper, minimal bacterial extrusion occurred with an increase in taper from 0.04 to 0.06 irrespective of the type of technique for root canal preparation. However, future studies are required for better exploration of this field of dentistry.

CONCLUSION

Significantly less amount of extrusion of bacteria is associated with 0.02 taper preparations when done with CD technique. No significant change in the amount of extruded bacteria occurs with an increase in taper from 0.04 to 0.06 irrespective of the type of technique for root canal preparation. However, future studies are required for better exploration of this field of dentistry.

REFERENCES


