Effect of different irrigating solutions on dentin microhardness at the furcation area of mandibular molars

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Abstract
The aim of the study is to evaluate the effect of EDTA, sodium hypochlorite and chlorhexidine on dentin microhardness at the furcation area of mandibular molars. 40 extracted human permanent mandibular molars were selected for study. Access opening and instrumentation done till No.F2 hand protaper file. Saline is used as irrigation during cleaning & shaping. Teeth were randomly divided to 4 groups (n=10) according to the irrigating techniques as Group 1: Saline, Group 2: ethylenediaminetetraacetic acid (EDTA), Group 3: sodium hypochlorite, Group 4: chlorhexidine. Irrigating solution were activated in the canals with manual activation technique. Then teeth were sectioned longitudinally approximately 5 mm below cementoenamel junction and embedded in acrylic resin block. Teeth were analysed for microhardness at furcation area with Knoop indenter. The result is the average dentin microhardness at furcation area is significantly higher in teeth irrigated with Chlorhexidine compared to EDTA, and sodium hypochlorite.

Keywords: Dentin microhardness, Furcation area, EDTA, Sodium hypochlorite, Chlorhexidine.

1. Introduction
A successful endodontic treatment requires good shaping, removal of infected tissues, and 3D obturation of the canal. Use of appropriate irrigation agents and instruments are key to success of root canal treatment [1]. Berutti et al. stated that solutions used as irrigation agents could penetrate about 130 μm into the internal surfaces of root dentin [2]. However, bacteria may be present in root dentinal tubules upto 1000-μm depths. To achieve proper antibacterial effectiveness, irrigating agents should remove organic and inorganic tissues in root canals, open dentinal tubules, and penetrate into the root canal system [3].

NaOCl, EDTA and chlorhexidine are widely used irrigating agents in endodontics. However, long-term use of these agents at high concentrations can cause undesirable effects on the root canal walls, such as significantly reduced flexural strength, micro-hardness, root canal dentin roughness [4].

NaOCl is excellent antibacterial agent capable of dissolving necrotic tissue, vital pulp tissue and the organic components of dentin and biofilms. So it is irrigant of choice in endodontics. Chlorine is responsible for dissolving and antibacterial capacity of NaOCl. Efficacy of the irrigant depends on concentration, temperature and time [5].

Ethylenediamine Tetra Acetic Acid (EDTA) is a chelating substance. It is capable of removing calcium ions of the dentin, giving rise to demineralization and as a consequence, increasing the dentin permeability of the root canals [6].

Microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues as it depends on amount of calcified matrix per square meter [6]. Reduction in microhardness or physical properties of dentin can lead to decrease in fracture resistance of dentin at furcation area. This can cause fracture at furcation area. Thus it is important to know effect of these irrigating solutions on dentin microhardness at furcation area.

2. Materials and Methods
Fifty freshly extracted human permanent mandibular molars were taken for the study. Access opening was done in all the teeth followed by cleaning and shaping till No.F2 hand protaper file.0.5ml saline was used as irrigating agent after each instrumentation. Teeth were randomly divided into 4 groups according to irrigation regimens, where each group contain 10 samples. Saline is used as control group. And teeth were irrigated with 5.2% NaOCl, 17% EDTA and chlorhexidine.1 ml of each irrigating solution were used upto 10 mins in the canal. Final flush was done with 5 ml of distilled water.
After the treatments, teeth were sectioned longitudinally in mesiodistal direction. Surfaces of teeth were polished and mounted on acrylic. Microhardness of dentin was measured at furcation area using Vickers hardness test (Instron machine) using 50gm load and 20sec dwell time. Data were analyzed statistically by one-way ANOVA and Tukey’s multiple comparison test. A significance level of 5% was set for all analyses.

### 3. Results
The microhardness values (mean ± SD) for the irrigating regimens are summarized in Table.

<table>
<thead>
<tr>
<th>Irrigants</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>54.5 ± 8.3</td>
</tr>
<tr>
<td>EDTA</td>
<td>42.9 ± 5.6</td>
</tr>
<tr>
<td>Sodium Hypochlorite</td>
<td>45.9 ± 4.8</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>47.9 ± 7.9</td>
</tr>
</tbody>
</table>

After the treatments, teeth were sectioned longitudinally in mesiodistal direction. Surfaces of teeth were polished and mounted on acrylic. Microhardness of dentin was measured at furcation area using Vickers hardness test (Instron machine) using 50gm load and 20sec dwell time. Data were analyzed statistically by one-way ANOVA and Tukey’s multiple comparison test. A significance level of 5% was set for all analyses.

### Discussion
In this study, NaOCl has been used as an endodontic irrigant for the chemomechanical preparation of root canals because of its excellent antimicrobial action, capacity of dissolving organic tissue remnants and improving the action of instruments by lubricating the canal walls.

Chlorhexidine is an efficient antimicrobial agent that has encouraged its use in endodontics alone or as an adjunct to sodium hypochlorite. Its antimicrobial effectiveness has been confirmed by different studies in vivo. Chlorhexidine has the advantage of adsorbing to dentinal wall and this provides an important residual antimicrobial activity in the root canal.

Chelating agents such as EDTA have been the irrigant of choice to demineralise dentin and aid in removal of organic smear layer. Therefore, in the present study, 5.2% NaOCl, 17% EDTA and chlorhexidine were used to evaluate the effect of these irrigating regimens on dentin microhardness at the furcation area. Microhardness determination can provide indirect evidence of mineral loss or gain in the dental hard tissues [7].

In this study, EDTA showed more reduction in dentin microhardness. This may be because of chelating effect of EDTA. EDTA binds to calcified components (particularly Ca+2 ions) of dentin through chelation action; thus, in turn, it causes demineralization and softening of dentin. The chelating action of EDTA solution induces an adverse softening potential on the calcified components of dentin and consequently a reduction in the microhardness was expected. The demineralizing effect of EDTA with consequent decrease of microhardness has been extensively demonstrated in various studies [8].

NaOCl showed significantly less dentin microhardness when compared to control group, it may be due to its property of dissolution of organic component. NaOCl is known to fragment long peptide chains and to chlorinate protein terminal groups, the resulting N-chloramines are broken down into other species. Thus affecting mechanical properties of dentin by degradation of organic components of dentin. Kinney et al. [9] suggested that the decrease in hardness is caused by a decrease in stiffness of intertubular dentin matrix caused by heterogeneous distribution of the mineral phase within the collagen matrix. Slutzky-Goldberg et al. [10] found that root canal irrigation with 2.5% and 6% NaOCl reduced dentin microhardness at a depth of 500 μm from the canal lumen. According to those authors, both the concentration and the irrigation period affect the action of NaOCl in reducing microhardness. Oliveira et al. [5] stated that the use of 1% NaOCl for 15 min can reduce dentin microhardness at depths up to 1,000 μm from the canal lumen.

Chlorhexidine had the least effect on dentin microhardness when compared to EDTA and NaOCl, but it showed some alteration in dentin microhardness than Saline group. Chlorhexidine gluconate widely used as endodontic irrigating solution it has antimicrobial effect at 0.2%. Chlorhexidine has direct effect on components of dentin structure disrupting the links between collagen fibers and hydroxyapatite crystals causing decrease in dentin microhardness. Chlorhexidine is cationic molecule structure because of this property Chlorhexidine has residual effect [11]. Ferraz et al in a clinical study showed that 2% Chlorhexidine produced cleaner and smoother root canal surface thus suggesting that it had some softening effect on root dentin thus supporting our observation [12].

Reduced dentin microhardness could be beneficial under clinical conditions as it would allow rapid preparation and negotiation of tight root canals. However, the degree of softening and demineralization will affect the physicochemical properties of this heterogenic structure [9]. Previous studies have shown that some procedures such as removal of dental tissue during instrumentation [8], canal space preparation to receive intraradicular retainers and use of chemical substances may increase the susceptibility to vertical root fracture. Kruzic and Ritchie [13] reported that the destruction of the collagen matrix in dental hard mineralized tissues results in a less tough, more brittle substrate that might precipitate fatigue crack propagation during cyclic stresses.

From the results of the present study, it may be concluded that 17% EDTA, reduced significantly dentin microhardness at the furcation area in mandibular molars. Therefore, care should be taken during chemomechanical preparation of these teeth, especially those with weakened furcation area.
5. Conclusion
The average dentin microhardness at furcation area is significantly higher in teeth irrigated with Chlorhexidine compared to EDTA, and sodium hypochlorite.

6. References