Comparative Evaluation of Smear Layer Removal by Apple Vinegar Using Laser Activated Irrigation and EndoVac System: Randomized Control Trial

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ABSTRACT

Purpose: This study was designed as randomized control trial to assess the effect of laser activation versus EndoVac systems with 5% apple vinegar irrigant on smear layer removal from root canal wall using scanning electron microscope. Material and Methods: Thirty three single rooted teeth were selected and prepared using Universal ProTaper rotary files and irrigated using sodium hypochlorite. Samples were randomly alienated into three groups (11 samples each) in proportion to method of activation of apple vinegar which used as final irrigant: Group I: Laser activated irrigation using 810nm diode laser at 1.5 Watts power in pulsed mode, Group II: EndoVac system used according to manufacturer instruction, and Group III (Control group) irrigating needle was used for irrigation, the amount of irrigant and time of application were adjusted according to each irrigating method. Scanning electron microscopic analysis was performed to evaluate smear layer of each group at different root canal levels. Results: No significant difference between tested groups in coronal and middle thirds but there is significant difference in apical third and total. In apical third, there is statistically significant difference between laser activated and EndoVac compared to control group, while no significant difference was found between EndoVac system and laser activated group (P-value <0.05). Conclusion: This in-vitro study demonstrated that using of 810 nm diode laser activation and EndoVac system with apple vinegar are effective in removing smear layer compared to conventional irrigation, particularly at apical third of root canal system.
INTRODUCTION

Successful endodontic treatment attainment relies on removing pulp remnants, microbial toxins and microorganisms which might be achieved during the chemo mechanical preparation. As a result of the instrumentation, the accumulation of debris occurs on the canal wall forming a heterogeneous amorphous layer named smear layer that formed of both; organic and inorganic components\(^1\). Although there is controversy about retaining or eliminating the smear layer, recently it was reported that presence of this layer interfere with the entrance of intracanal medicaments inside the dentinal tubules and prevents close adaptation of obturation materials to the walls of root canal; thus smear layer removal was recommended to ensure the long-term success of root canal treatment\(^3\).

Smear layer removal can be achieved through using efficient irrigating and chelating solutions to eradicate both the inorganic and organic components. Sodium hypochlorite (NaOCl) is the most regularly used irrigating solution, due to its efficiency in tissue disintegration and antibacterial property, thus it is effective in organic part removal\(^4,5\). Ethylenediaminetetraacetic acid (EDTA) is a chelating agent able to eliminate smear layer’s inorganic part, EDTA used alternatively with NaOCl which is recommended irrigation protocol to clean the smear layer completely, but this technique has detrimental effect on dentine mechanical properties and cause intertubular dentine erosion\(^6,8\).

Natural irrigants have been introduced to minimize the damaging effect of chemical irrigating components on dentin and being biocompatible to the periapical tissue. Apple vinegar is natural solution produced through fermentation process, it is composed of different acids, however the acetic (5%) and malic (0.35%) acids are two highest acids concentration of the vinegar\(^9,10\). It was proven to be a highly biocompatible irrigant, has antimicrobial effect against different microorganisms associated with endodontic infection as Enterococcus Faecalis, and be efficient in smear layer removal without deleterious effect on calcium content of interradicular dentine\(^11,12\).

Passive irrigation technique using irrigating needle with different tip design is most popular method applied in endodontic preparation. It was reported that this conventional technique being less efficient in smear layer removal particularly in curved canal and apical third of the root canal system, not able to remove vapor lock effect and may cause over irrigation\(^13-15\). Irrigation activation techniques was established to improve the efficiency of root canal cleaning and overcome deficiency of conventional syringe irrigation, thus recent irrigation systems had been introduced to improve the mechanical flushing action of irrigants, with better removal of smear layer from anatomical complexities and dentinal tubules\(^6,16\).

The EndoVac System (EV) is an apical negative pressure irrigation system introduced to provide efficient clear out the root canal system specially the curved canals, deliver irrigating solution 1-2mm apically without risk of solution extrusion and overcome the apical vapor lock effect. In this system irrigating solution delivered to pulp chamber by needle and negative apical pressure produced by fine suction tip seated near the working length\(^18\). It was reported that EV system was helpful in debris and smear layer removal at different root canal levels, showed less debris extrusion and confident results about cleaning and debridement of root canal system\(^19-21\).

Laser application in endodontics such as carbon dioxide (CO2) and neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers were proved to be effective in disinfection and cleaning of entire root canal system with high penetration depth into dental tissue\(^22,23\). After advance of laser application the diode laser has been introduced, it has a broad range of wavelengths, however the most suitable wavelengths for the intraoral application is between 940-980 nm. Other advantages of diode laser of
being affordable, portable, has thin flexible optical fiber that able to reach more depth in curved canal and different anatomical areas difficult to access also 810nm and 980nm diode lasers reported to demonstrate bactericidal effect \(^{(24,25)}\). Laser activated irrigation has become common application in endodontics, it promotes warming of irrigant beside agitating action which improve its effect, 810 nm diode laser was reported to be effective in removal of smear layer with EDTA solution \(^{(24,26)}\).

This study was designed as randomized control trial to assess the effect of laser activation versus EndoVac systems with 5% apple vinegar irrigant on smear layer removal from root canal wall using scanning electron microscope.

Null hypothesis of this study was that there is no difference between laser activated irrigation and EndoVac system of apple vinegar on smear layer removal.

**MATERIAL AND METHODS**

**Samples size calculation:**

Sample size was calculated at 80% power of the study and 95% confidence level by the statistical formulas, so 11 subjects were selected for each group \(^{(27,28)}\).

**Teeth selection:**

Thirty three extracted human maxillary and mandibular single rooted teeth (which are anonymous teeth) with Vertucci’s type I canal configuration and fully formed root apices were assessed visually and radiographically for absence of root caries and root cracks or previous root canal fillings were randomly selected for this study. Ethical approval for the use of extracted human teeth was obtained in accordance with guidelines from Research Ethic Committee (REC), Faculty of Dental Medicine for Girls, AL-Azhar University, Code (REC-EN-21-06).

**Specimens’ preparation:**

After soft debris and calculus removal all teeth were disinfected and stored in sterile saline. Proper access cavity was performed using round and safe end cutting bur. Working length (WL) was determined using a #10 K-file (MANI Inc., Japan) which was adjusted by subtracting 1mm after observation of file tip at root apex. Preparation of root canal system was performed using ProTaper Universal rotary NiTi files (Dentsply, Mailfer, Switzerland) that were used in a crown-down manner, with torque and speed adjusted according to manufacturers’ recommendations for each file used. Copious irrigation was applied after every file with 2 ml 2.6% NaOCl solution (Alex. Detergents and Chemical Co., Egypt) for 1 minute dispensed through a 31-gauge Navi-Tip flexible irrigating needle (Navi-Tip, Ultra- dent product, South Jourdan, UT).

**Specimens’ grouping:**

After root canal preparation thirty three teeth were divided randomly into three groups (11 samples each) depending on the method of irrigation activation of 5% apple vinegar which was used as final rinse. Question in this study was addressed in terms of PICO question which involves 4 elements: [problem (P), intervention (I), comparison (C) and outcome (O)] as following:

- **P.** Smear layer formation (problem).
- **I.** Activated irrigation with apple vinegar (intervention).
- **C.** Laser Activated Irrigation versus EndoVac system (comparison).
- **O.** Removal of smear layer (Outcome).

**Group I:** Laser Activated Irrigation; 810nm diode laser using 1.5 Watts power in pulsed mode in 4 applications of 5 seconds with 20-sec intervals in between, fiber optic tip was inserted 1 mm from WL and moved in slow helical motion from the apex to the cervical third with alternating between clockwise and counterclockwise direction.
0.8ml of 5% apple vinegar was used for each application and the rest was 0.2 ml, after that the root canal was conventionally irrigated with 10ml of distilled water. Group II: EndoVac (EV) system; one macroirrigation cycle applied using 5 ml of 5% apple vinegar for 30 sec performed using the master delivery tip and the macrocannula, followed by 60 sec of passive wait. Another three cycles of microirrigation in which of each 5 ml was used for 30 sec of active irrigation, the microcannula was continually moved up and down in the canal ( 2mm from WL) while the pulp chamber was kept full of irrigating solution, followed by 60 sec in which the irrigant was left undisturbed. At the end of the third cycle, the microcanula was left at the working length to remove excess solution, and then the root canal was irrigated with 5ml distilled water and dried using paper points. Group III: needle irrigation (Control group); the root canal system was irrigated with 5 ml of 5% apple vinegar using a 31-gauge side vented Navi-Tip flexible irrigating needle (Navi-Tip, Ultradent product, South Jourdan, UT) for 1 minute followed by 10 ml of distilled water.

**Scanning electron microscopic (SEM) evaluation:**

The specimens were prepared for SEM examination by splitting vertically into two halves, guided grooves were made longitudinally at buccal and lingual surface using diamond disc and separated with chisel to reveal the internal canal wall. A caliper was used to measured length of root canal from cementoenamel junction (CEJ) to the apex to determine the three thirds (coronal, middle, and apical). Specimens were mounted on metallic stump using sticky glue and digital photomicrographs were taken under magnification (X 1000) at each third for evaluation of smear layer to analyze by means of numerical evaluation score.

Smear layer scoring was performed blindly by two endodontists who had no knowledge about study design. The evaluation was done using Hulsmann score (29), a five-point scoring system.

Score1: no smear layer and dentinal tubules widely opened. Score2: a little quantity of smear layer and some dentinal tubules open. Score3: homogeneous smear layer coating the root canal while just a few dentinal tubules open. Score4: entire root canal coated by a homogenous smear layer and no open dentinal tubules. Score5: intense, non-homogeneous smear layer coating the root canal surface completely.

**Statistical analysis:**

Nonparametric Kurskal Wallis was used to compare groups in each root level while Post hock test (Mann Whitney U test) used to show the significant between each two groups in apical and total. P-value <0.05(*) was considered significant difference & P-value <0.001(**) was considered highly significant difference.

**RESULTS**

Descriptive statistics expressed using median, minimum and maximum of all tested groups were represented in table (1). Nonparametric Kurskal Wallis was used to compare smear layer scores of tested irrigation techniques at all root canal levels showed that there was a statistically significant difference between the groups at all root canal level (P-value <0.05). Post hock test (Mann Whitney U test) revealed that there was statistically significant difference between laser activated irrigation (group I) and control group (group III), and also between EndoVac system (group II) and control group (P-value <0.05), while there is no statistically significant difference between laser activated irrigation and EndoVac system groups, both showed less score level of smear layer than needle irrigation technique group that revealed better removal of smear of those techniques with apple vinegar irrigation solution.

Regarding the smear layer score at each root canal levels (coronal, middle, and apical), there is no
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statistically significant difference between the three groups at coronal and middle thirds but there was statistically significant difference between them in apical one third (P-value <0.05). At the apical third level, there is a significant difference between laser activated group and control group, and also between EndoVac system group and Control group. But there is no significant difference between EV system group and laser activated group. These results proved effectiveness of using laser activation and EV system in removal of smear layer at apical part of the root canal than using conventional irrigating needle with apple vinegar irrigating solution (Fig. 1 and 2).

Table (1): Descriptive analysis of smear layer score at coronal, middle & apical root levels of all the studied groups.

<table>
<thead>
<tr>
<th>Root Level</th>
<th>Group I Laser activated irrigation</th>
<th>Group II Endo Vac system</th>
<th>Group III Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Median</td>
<td>Min</td>
</tr>
<tr>
<td>Coronal</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Middle</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Apical</td>
<td>1</td>
<td>3</td>
<td>2A</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>3</td>
<td>2A</td>
<td>1</td>
</tr>
<tr>
<td>P-value</td>
<td>0.764</td>
<td></td>
<td>0.092</td>
<td></td>
</tr>
</tbody>
</table>

There is a significant difference at p-value <0.05(*), highly significant difference at p-value <0.001(**). The groups with the same letter: there is no significant difference between them and groups with different letter: there is a significant difference between them.

Figure (1) A bar chart comparing the median scores of smear layer among laser activated irrigation, EndoVac system and needle irrigation techniques groups at the coronal, middle & apical levels of the root canal.
DISCUSSION

Smear layer formed during root canals instrumentation consists of dentin chips, bacteria and their byproducts, microorganisms, and tissue remnants \(^{30}\). This layer coats root canal dentinal walls which preserves bacteria in the dentinal tubules, interferes with deep penetration of irrigating solution and root canal sealer, prevents adaptation between obturation material and root canal wall that can disturb the apical seal which subsequently affect success rate of endodontic treatment \(^{31,32}\). Sodium hypochloride (NaOCl) which has capability to remove organic components used alternatively with EDTA chelating agent concerned with inorganic part were used to remove smear layer completely, however this comments irrigation protocol has detrimental effect on dentinal structure\(^{4,33}\). Recently using of more biocompatible and dentin friendly irrigating natural solution is needed, accordingly apple vinegar which was reported to had antimicrobial property, biocompatible, has chelating action and efficient smear layer removing irrigant has been used in study to eliminate the smear layer \(^{9,12,34}\).

Conventional needle irrigating technique was reported to be less efficient in smear layer removal particularly in apical part of curved root canals, and different anatomical areas of the root canal system \(^{15}\). Recently newer irrigation systems which improve the mechanical action of irrigants with better smear layer removal were introduced. The EndoVac system (EV) which is an apical negative pressure irrigating system was introduced, it was
reported that EV provides efficient removal of smear layer within different areas of canal system, curved canals and the apical part of root canal without irrigation extrusion from apical foramen \(^{20,21,15}\).

Laser-activated irrigation used in cleaning procedures in endodontics showed promising results in smear layer removal which is achieved by irrigant activation with decontamination of the canal system simultaneously \(^{36}\). The laser diode has wavelength of 810 nm within the infrared range was recommended for endodontic treatment, it has bactericidal effects, has capacity to eliminate organic tissue as well as smear layer from the wall of root canal, open the dentinal tubules, and fuse hydroxyapatite \(^{24,25}\). Current study was designed to compare effect of laser activated irrigation versus EV system with apple vinegar on smear layer.

This study was designed as randomized control trial in which samples selection was randomly allocated into three groups depending on technique of apple vinegar irrigation activation. Also scoring of the smear layer was performed blindly by two endodontists other than the operator who designed the study and prepared the tested samples to decrease probability of bias.

This study results demonstrated that samples treated with 810 nm diode laser agitation with 5% apple vinegar (group I) provide better removal of smear layer than using of needle irrigation technique particularly at apical third. These results can be attributed to flushing action on solution caused by laser beam; also warming effect of laser radiation on irrigating solution that can improve the action of apple vinegar. The thermal effect was controlled during laser activation in this study through using of pulsed mode, continuous movement of fiber tip and applied for 5 seconds with 20-sec intervals in between each application\(^{37}\). In addition; using of flexible thin fiber laser tip that can reach to the narrowest area of the root canal up to 1mm of the apical constriction causes irrigation activation at this area. However, this technique is difficult to be applied with conventional needle irrigation to avoid solution extrusion from the apical foramen\(^{30}\). These results were in agreement with a study that applied diode laser irrigation activation on maleic acid which is the main component of apple vinegar\(^{38}\).

In group II using EndoVac system for apple vinegar activation resulted in less score level of smear layer was observed compared to conventional technique at all root canal levels with statistically significant difference especially at the apical third of the canal system. This result can interpret effective role of negative pressure technology used for irrigating solution delivery inside the root canal system which allows safe irrigation at 1-2mm of WL without risk of irrigant extrusion, moreover using of micro cannula that can be delivered to the apical third of the canal easily can enhance smear layer removal than conventional needle\(^{21}\). The results of this study were in accordance with study showed effect of EV improvement of irrigant on smear layer removal but other study used EDTA instead of apple vinegar\(^{20}\).

With regarding comparison between group I and group II, this study results showed no significant difference between the tested activation technique with apple vinegar, and both techniques showed low scores level of smear layer which indicates the success of both laser activated irrigation and EV system in cleaning the root canal system.

**CONCLUSION**

Within the limitations of this study the following could be concluded: Both 810 nm laser activated irrigation and EndoVac system improved action of 5% apple vinegar irrigating solution in smear layer removal at different areas of the root canal particularly apical third.

**ACKNOWLEDGEMENT**

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CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

RECOMMENDATION

Further studies are needed to investigate the effect of laser activated irrigation used in curved canals. Also, assessment of the effect of laser activated irrigation using apple vinegar on other parameters such as dentin micro-hardness, surface roughness and calcium content could be helpful.

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