Journal of University of Duhok, Vol. 22, No.1, 2019 (Pure and Eng. Sciences), Pp 30-37,(special Issue)
The 2nd international dental conference,28-29/3/2019, Duhok
https://doi.org/10.26682/sjuod.2020.22.1.4

COMPARTIVE EVALUTION OF A NEW ENDODONTIC IRRIGATION SOLUTION –APPLE VINEGAR, GINGER OIL AND SODIUM HYPOCHLORITE TO REMOVE THE SMERA LAYER BY SCANING ELECTRON MICROSCOPE STUDY

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(Accepted for Publication: August 29, 2019)

ABSTRACT

Introduction: A three dimensional seal is the one of main steps in the successful root canal treatment .The smear layer that has been created on the dentinal wall during root canal instrumentation should be removed by using irrigation solution . Irrigation solution should be always used with mechanical preparation of root canal system as an important part for successful endodontic treatment

Aim: The aim of this in vitro study was to compare the efficacy of four irrigating solutions in removing the smear layer.

Materials and Methods: A total of 40 single rooted teeth were selected and instrumented and then assigned in a random manner into 4 groups of 10 each. Each group treated with different solutions (Normal saline, Sodium hypochlorite, Apple vinegar and Ginger oil). Scanning Electron Microscope had been used to measure the effect of these materials (solutions) in removal of smear layer from three root sections (Apical, middle and coronal third of the root).

Conclusion: According to the Torbinjad criteria; Apple vinegar showed the best result in smear layer removal for the whole root length and no single irrigant can accomplish all the tasks required by irrigation.

KEYWORDS*: *Smear layer,Apple vinegar,Ginger oil,Irrigation

2. INTRODUCTION

" Endodontics" has stepped into the arena where more and more people are now realizing that saving even an isolated natural tooth is worth the time and effort as there is no substitute for a healthy natural dentition in order to maintain the integrity of the arch, function and esthetics of the masticatory apparatus.

The key objectives of endodontic therapy are cleaning and shaping, obturation of the root canal system in three dimensions and preventing reinfection. Cleaning and shaping are considered to be the most important and most demanding aspect. There is an old age saying in endodontics that is relevant even today "what is taken out of the root canal may be more important than what is put into the root canal."[1]

Irrigation solutions that used for irrigation of root canal have proved to be very important in root canal treatment, as they help in lubrication, disinfection, debris removal, and dissolving both organic and inorganic tissue from the root canal system [2]

So that, irrigation procedure is considered as an important step of root canal cleaning and disinfection .because complete debridement and disinfection cannot be achieved only by instrumentation.. In addition to disinfection, irrigants can also help remove the smear layer from the radicular wall. [3]

A large number of substances have been used as root canal irrigants, including acids (citric and phosphoric), chelating agents (EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea and potassium hydroxide), oxidative agents (hydrogen peroxide and Gly-Oxide) [4]. Apple vinegar has been tested by researchers in the field of dentistry as a chelating agent [5], Apple cider vinegar is used in a wide number of health-related issues such as in cancer, cardiovascular diseases, body and joint pains, diabetes, and weight loss. Its antimicrobial action is mainly due to the presence of acetic acid in it, that is, it causes loss of cell integrity. This also can be used in dentistry as a potent root canal

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irrigant. Very few studies have been carried out using apple cider vinegar as a potential root canal irrigant. [6]

Accurate debridement of root canals is recommended in most endodontic treatment.[7] Currently, various methods have been introduced to remove the smear layer which includes chemical, ultrasonic, and laser techniques, neither of them has been accepted universally nor they have proved to be more operative.[8]

Studies have been conducted with the aim of using chelating agents that would be more efficient and biocompatible with the organic structures than EDTA. Therefore, citric acid [9] and apple vinegar [10] have been studied. Apple vinegar has proven antimicrobial action, reduces dentinal microhardness, in addition to removing the smear layer. [11]

3. MATERIALS AND METHODS

Recently fourty extracted non-carious human mandibular and maxillary single rooted teeth obtained from patients 13- 60 years old were stored in saline at room temperature. The criteria for selection were length, straightness, and the apical morphology. If the apex was open to over # 20 K-file in diameter, the tooth was rejected and not used in the study. Teeth with average root length of 14-16 mm were selected. A total of 40 teeth were selected and assigned in a random manner into 4 groups of 10 each. [12]

The root surfaces of teeth were debrided and placed in 3% sodium hypochlorite solution for 24hrs to remove any remaining organic tissue. The teeth were stored in normal saline till the beginning of the study. After preparing conventional access cavity, the working length of all the teeth was established by passing a no. 10 file to the apical foramen and then reducing the length by 1 mm. The apical portion of the root tip was covered with sticky wax. [5]

Different types of irrigation fluids had been used. Irrigation solution which is normal saline considered as a control group, 30-gauge needle with side vent a blunt distal end was then attached to the syringe for irrigation. 5% NaOCl and normal saline were used.

The irrigations that are used in this study are (Normal saline (as control), 5% sodium hypochlorite, 95% apple vinegar, and 100% ginger oil).

- 5% of sodium hypochlorite prepared by mixing of 95ml of normal saline with 5ml of sodium hypochlorite at home. [12]
- 95% apple vinegar contains 5% sodium metabisulfied and 95% apple vinegar.

Preparation of the root canal:

The biomechanical preparation was done by using protaper (X Smart) densply rotary instruments in hand piece speed 300 torque 3.0 gear 16:1 up to the apical size F2 was used by crown down technique following the manufacturer instruction. SX used with brushing motion for orifice widening and coronal shaping, after that we used shaping file S1 with brushing action for preparing 2/3 of the length of the canal and then repeating using S1 for the whole length of the root. While S2 used for shaping the full working length of the root. Preparing was finished with finishing files F1, F2 for the full working length of the canal.

The canals were irrigated with 1 ml of either sterile saline solution or sodium hypochlorite (5%) after use of each instrument, according to the groups.

A volume of 10 ml volume of irrigant was used 5ml as initial irrigation during root canal preparation, and 5ml of test solution as a final solution for removal of smear layer. The irrigant was delivered with a 30-gauge, 1½ inch needle (ProRinse, Densply). The specimens were then divided into four groups, depending upon irrigant/irrigants used as a final rinses as shown in **Table 1**.

Table (1): Study Group

Group (n= 40)	Irrigating solution during root canal preparation (5ml)	Final solution for removal of the smear layer (5ml)
A	Saline	Saline
B	5% Naocl	5% Naocl
C	5% Naocl	Apple vinegar
D	5% Naocl	Ginger oil

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After completion of canal preparation till apical size F2, the crowns of all the teeth were removed at the Cemento-enamel junction with separating disks with coolant.

Final rinsing of root canals was done with the test solutions in the following manner:

With the help of 30-gauge Pro Rinse probe, 1 ml of the test solution was delivered in the canal as near as possible to the apex without binding. Test solution was left in the canal for 5min with in-between agitation by # 15 K-file, followed by remaining 4 ml irrigation. Final irrigation of root canals was done with 3ml of distilled water to remove any precipitate that might have formed from the test irrigants and the canals were dried with paper points.

Scanning Electron Microscope Analysis:

Horizontal grooves were made on both buccal and lingual surfaces of the root using a diamond disk without penetration of the canal. With the help of a chisel, the roots were then separated into two halves. The half portion of each root was coded and chosen, containing the most visible part of the apex and the whole canal length.[13] **Figure1**. One half of the root was discarded and the other half was placed in 2% glutaraldehyde solution for 24hrs.

Fig(1): Half of the root

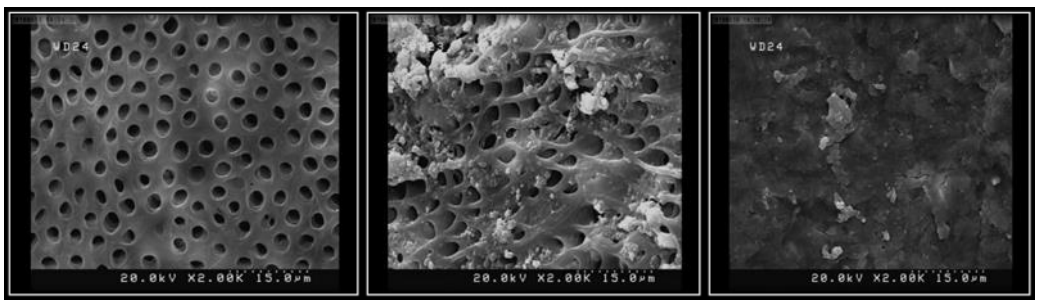
The root surface was divided into three parts (apical, middle, coronal) by using fixed marker. The samples then sent for scanning electron microscope examination and taking photomicrograph for these three parts. The coded and mounted samples were placed in the vacuum chamber of the SEM. The acceleration voltage was standardized to 7 and 20.0 K.V with an emission current of 15.0 µA and width of 20 mm. The angle of tilt and the aperture was adjusted to optimize the quality of photomicrograph.

Each sample was micro-graphed at these three areas (apical, middle, coronal) at different magnification and viewed under a SEM. The photomicrographs taken were qualitatively evaluated blindly by three evaluatores, and rated for the degree of cleanliness (**Figure2**) with regard to the presence of debris, smear layer and patency of dentinal tubules[14] on a scale of 1 to 3 where:

1 = No smear layer. Clean and open tubules, the surface of the root canals free of the smear layer.

2 = Moderate smear layer. The surface of the root canals free of the smear layer, but debris found in tubules.

3 = Heavy smear layer. The root canal surface and the tubules covered by the smear layer.



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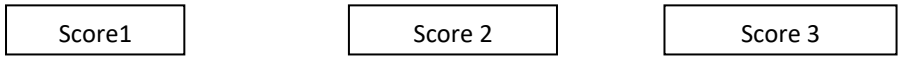

Fig. (2): SEM scores

- **Statistical analysis:**

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Kruskal Wallice test was used to compare the mean rank of the smear layer removal of the study groups. A p value of ≤ 0.05 was considered statistically significant.

4. RESULT

According to the parameters of smear layer removal by type of materials (solutions) and root sections. Apple vinegar showed the least mean value for the three root sections (Apical, Middle and Coronal) (1.40, 1.00, and 1.40 respectively). Followed by Sodium hypochlorite with low mean values for both middle and coronal third of the roots (2.00). While Normal saline and Ginger oil showed the highest mean values with (at) different sections. The mean value of the apical and middle third of the roots were (was) (3.00) for normal saline. While Ginger oil showed the mean value of (3.00) for both middle and coronal third.

According to the Torbinjad criteria Apple vinegar showed the best result in smear layer removal for the whole root length.

Table (2): Parameters of smear layer removal by type of material and section.

		Smear layer removal			
Material	Section	Mean	Median	Minimum	Maximum
Group 1	Apical third of the root	3.00	3.00	3	3
	Middle third of the root	3.00	3.00	3	3
	Coronal third of the root	2.40	2.00	2	3
Group 2	Apical third of the root	2.40	2.00	2	3
	Middle third of the root	2.00	2.00	2	2
	Coronal third of the root	2.00	2.00	2	2
Group 3	Apical third of the root	1.40	1.00	1	2
	Middle third of the root	1.00	1.00	1	1
	Coronal third of the root	1.40	1.00	1	2
Group 4	Apical third of the root	2.40	2.00	2	3
	Middle third of the root	3.00	3.00	3	3
	Coronal third of the root	3.00	3.00	3	3

From Table 3 it is obvious that significant differences were detected between the four groups regarding the mean rank of smear layer removal by type of material for each root section at a ($P < 0.001$). Least mean ranks were detected with Apple vinegar for the three sections (Apical, Middle, and Coronal) (7.90, 5.50, and 8.70) respectively. While the highest value (33.50) were was detected with ginger oil at the coronal third of the root.

Table (3) :(Kruskal Wallice test**) Smear** layer removal by type of material, in each of the root sections.

		Mean rank of smear layer removal					
Material	N	Apical third only		Middle third only		Coronal third only	
NS	10	31.50		30.50		23.30	
Sodium h.	10	21.30	< 0.001	15.50	< 0.001	16.50	< 0.001
Apple V.	10	7.90		5.50		8.70	
Ginger oil	10	21.30		30.50		33.50	

For all used solutions except Apple vinegar, the statistical analysis showed significant difference in the mean rank of smear layer removal at P value < 0.05 (Table 4).

Considering the Normal saline, the table show significant differences in the mean rank of smear layer removal by section of the tooth P value (0.001). It's evident that least mean value (9.50) was detected on the coronal third of the root. Also its evident there is no difference between apical and middle third of the root that equal to (18.50) (table 4).

Sodium hypochlorite and Ginger oil also showed a significant differences at a P value

https://doi.org/10.26682/sjuod.2020.22.1.4

(0.012, 0.001) respectively. It's evident no difference between the coronal and middle third of the root for each material with a mean rank value (13.50, 18.50) respectively. While in the apical third of the root, sodium hypochlorite showed the higher mean value (19.50) but Ginger oil showed the lowest mean value (9.50).

While Apple vinegar was the only material that showed non-significant differences by section of the tooth with P value (0.072), with the least mean value (11.5) at the middle third and higher mean value at both apical and coronal third of the root with mean value (17.50).

Table (4): :(Kruskal Wallice test) Mean ranks of smear layer removal by section of the tooth, in each type of the materials.

Material	Section	N	Mean Rank	P
NS	Apical third of the root	10	18.50	
	Middle third of the root	10	18.50	0.001
	Coronal third of the root	10	9.50	
Sodium	Apical third of the root	10	19.50	
	Middle third of the root	10	13.50	0.012
	Coronal third of the root	10	13.50	
Apple	Apical third of the root	10	17.50	
	Middle third of the root	10	11.50	0.072
	Coronal third of the root	10	17.50	
Genjour oil	Apical third of the root	10	9.50	
	Middle third of the root	10	18.50	0.001
	Coronal third of the root	10	18.50	

5. DISCUSSION

Using irrigation solutions in root canal therapy is an essential procedure for the removal of the smear layer. The clinical use of new irrigation and chelating solutions must be preceded by laboratory studies that investigate the benefits and consequences to the human beings.[5] Removing the smear layer enhances disinfection into dentinal tubules in addition to allowing tridimensional sealing of the root canal system.[9] Irrigation with a tissue dissolving antimicrobial solution is a prerequisite for effective removal of the smear layer and remnant pulp debris which may in turn affect sealing ability of filling materials.[15]

This is because Apple vinegar is constituted of acetic acid (its main component), malic, lactic, formic, and citric acids. Malic acid is the constituent responsible for the therapeutic property of the solution.[9] The presence of malic acid gives the biocompatibility action to apple cider. In this process, the ethyl alcohol produced is converted and oxidized into acetic acid under the presence of specific microorganisms. This procedure is called acetification. [16] The total amount of calcium ion found in the apple vinegar solution is due to the action of H+ ions present. The more the concentration of H+ ions the more efficient the attack of the acid would be.[13] Furthermore, the apple cider vinegar has a medicinal potential due to its rich mineral content such as potassium, phosphorus, and magnesium. Despite fully knowing its mechanism of action, it is believed that adsorption, ionic exchange and chelation are responsible for the elimination of dentin calcium ions. [11] Apple vinegar has proven antimicrobial action, reduces dentinal microhardness,[10] in addition to removing the smear layer. [17],[18]Apple vinegar associates a good capacity to remove smear layer from the dentinal tubule entrances with bactericidal action against microorganisms that are frequently associated with endodontic infections, such as ***Staphylococcus aureus*** and ***Enterococcus faecalis***. The high biocompatibility of apple vinegar is mainly attributed to the high concentration of malic acid in its composition. [19]

Estrela et al. 2007 [10] assessed the smear layer removal capacity of apple vinegar used in isolation and/or associated with EDTA and they observed that the action of apple vinegar in removing the smear layer may be increased when EDTA is associated with the solution. The result of this study also agree with George et al , 2011[19], they assessed that the apple vinegar associated or not with EDTA was more effective in removing smear layer from the root canals than NaOCl associated with EDTA.

The results of this study also showed that there were no statistically significant differences as regards the different root thirds or sections (coronal, middle, and apical) for the apple vinegar, being in accordance with the studies by Scelza et al. 2004[18] This is due to the methodology applied, in which the solution used had free passage through the root canal, homogeneously promoting wettability of the root dentin.

The second irregant that shows the best result after the apple vinegar was sodium hypochlorite for the coronal and middle third part of root. Sodium hypochlorite is the most popular root canal irrigant currently used. It is popularity is due to its tissue dissolving property along with being antimicrobial and potent lubricant.

Sodium hypochlorite has a very high pH which effect on the cytoplasmic integrity with an irreversible enzymatic inhibition, biosynthesis alteration in cellular metabolism, and phospholipids degradation. NaOCl is a popular irrigant for the excellent lubricant action and broad spectrum of antibacterial activity and its capacity to dissolve organic tissue. It has also been suggested that higher the concentration, the better the antibacterial and tissue dissolution properties.[20] However, along with its many advantages, it has many disadvantages including toxic and bad odor. Many cases of sodium hypochlorite accidents have been reported. Thus, many alternative irrigants have been researched over the years that will overcome sodium hypochlorite various drawbacks.[6] However, the scanning electron microscopic pictures of NaOCl in the study done by Vallabahaneni K et al, 2017 showed the absence of superficial debris with the presence of smear layer at all root thirds, signifying the inability of 5.25% NaOCl incomplete removal the smear layer.[21] These results were similar with Yamada RS et al, 1983 and Baumgartner JC et al, 1984, suggesting that 5.25% NaOCl was competent in removing organic and loose superficial debris, leaving exposed inorganic component of smear layer preventing its further removal. [22,23] However Hebatalla E.Kandil et al, (2014) stated that the NaOCl was an ineffective irrigant to remove the smear layer.[24]These findings are similar to those observed in previous investigations Torabinejad et al. 2003 [8], Ulusoy and Gorgul, 2011 [25], Mozayeni et al.2009 [26], that showed these irrigants are not able to remove both organic and inorganic components of the smear layer.

Ginger oil were showed the least effective material for removing smear layer especially at the coronal third of root when it compared to the other parts of the root. This is may be due to low chelating ability of this oil but it still show a good antimicrobial effect in another study. Hence, further research is required and more in vivo studies need to be done to evaluate these root canal irrigants in detail regarding its physical, chemical, biological and antimicrobial properties in order to verify the benefits and consequences to humans.

While normal saline was used in this study as a control group that shows the least effective material in removing smear layer especially at the apical and middle third of root.

Several studies have showmen, that mechanical preparation with manual instrumention and irrigation with saline cannot predictably eliminate the bacteria from the infected root canals[19] Hebatalla E.Kandil et al, (2014) stated that Specimens treated with NaOCl and saline showed thick smear layer in the three thirds of the root canals. [24]

Vemuri S et al, (2016) stated the normal saline was the least effective material in removing of smear layer in all the three parts of the root.[27]

The major advantage of these natural alternatives are their easy availability, low cost and most importantly their excellent bio-compatible nature with negligible side effects.[28]

6. CONCLUSION

Although apple vinegar show the best result as irrigating solution, no single irrigant can accomplish all the tasks required by irrigation. Detailed understanding of the mode of action of various solutions is important for optimal irrigation.

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پوختە

ئامانج: ئامانجی ئەم توێژینەوەیە بۆ بەراوەردکردنی کاریگەری چەند ماددەیەکی نوێ بۆ خاوێن کردنەوەی ناو دەماری ددانە لەکاتی دەمار بڕیندا .

ماددەکان وڕێگای بەکارهێنانیان: تێکرای چل ڕەگی ددان بەکارهێنرا پاش خاوێن کردنەوەی ناو ڕەگی ددان دابەشکران بەشێوەیەکی هەرەمەکی بەسەر چوار گروپدا هەر گروپێك پێکهاتبوو لە دە ڕەگی ددان. هەر گروپێك خاوێن کراوە لەگەل یەکێك لەو ماددە جیاوازانە (سرکەی سێو، ڕۆنی زەنجەبیل، کیراوەی سۆدیۆم هایپۆکلۆراید لەکەل نۆرمەڵ سەلاین). لە پاش بەکارهێنانی مایکرۆسکۆپی ئەلیکترۆنی بۆ پێوانەکردنی کاریگەری ئەو ماددانە لەسەر ناو ڕەگی ددان لە هەر سێ پارچەی ڕەگی ددان (بەشی سەرەوە و ناوەوە و خوارەوەی ڕەگی ددان).

دەرئەنجام: سرکەی سێو باشترین دەرئەنجامی پیشاندا لە خاوێنکردنەوەی هەر سێ پارچەی ناو ڕەگی ددان بەڵام هیچ کام لەم مادە بەکارهێنراوانە بە گوێرەی پێویست خاوێنکردنەوەی ناو ڕەگی ددانیان نەکردووە.

الخلاصة

الهدف: الهدف من هذه الدراسة المختبرية هي لمقارنة كفاءة اربعة محاليل تنظيف في إزالة طبقة اللوثة (اللطخة).

المواد وطرق العمل: تم اختيار ما مجموعه اربعين سن من الاسنان ذات الجذور الفردية وتم تنظيف قنواتها. ثم تم توزيعها بطريقة عشوائية في اربعة مجموعات كل منها تظم 10 سن. كل مجموعة تعامل بمحلول مختلف (محلول ملحي طبيعي، صوديوم هايبوكلورايت ، خل التفاح، و زيت الزنجبيل). تم استخدام مجهر المسح الضوئي الالكتروني لقياس تأثير هذه المحاليل في إزالة طبقة اللطخة (اللوثة) من الاقسام الثلاثة للجذر (الثلث القمي، الثلث الاوسط، والثلث الاكليلي) .

الأستنتاجات: وفقاً لمعايير توربنجات، أظهر خل التفاح أفضل نتيجة في إزالة طبقة اللوثة (اللطخة) لطول الجذر بالكامل، ولا يمكن لأي غسول منفرد إنجاز جميع المهام التي يتطلبها الغسل.