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The Influence of Irrigation with Different Solutions on Apical Microleakage of Root Canals

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Abstract

Aims: This study aims to assess the apical microleakage of root canals after irrigation by NaOCl, EDTA, and MTAD. **Materials and methods:** 36 lower premolars were used in this study. Preparation of the samples was done by files of ProTaper to size F3. Samples were distributed into three experimenting groups; each one contained ten samples as follows: Group 1- samples were finally washed for five minutes with five ml of 5.25% NaOCl. Group 2- samples were finally washed for five minutes with five ml of 17% EDTA. Group 3- samples were finally washed for five minutes with five ml of MTAD. Six samples were utilized as positive and negative control groups, each one contained three samples. The samples were obturated by single cone technique except for the samples of the positive control group, which were not obturated. All the samples were incubated at 37 ^{O}C for 7 days in 100% humidity. The dye penetration method was used to assess apical leakage. Finally, data was collected and statistically analyzed. **Results:** MTAD group revealed the least leakage supervened by the EDTA group and the NaOCl group revealed the highest leakage. The MTAD group did not differ significantly from the EDTA group, while the NaOCl group differed significantly from MTAD and EDTA groups. A group of positive control revealed a dye leak through the whole length of the root, while a group of negative control did not reveal any dye leak. **Conclusions:** Under the conditions of this study, the final irrigation with MTAD or EDTA is better than the final irrigation with 5.25 % NaOCl in terms of less apical microleakage.

الخلاصة

الأهداف: تهدف الدراسة الى تقييم التسرب المجهري القمي لقنوات الجذوربعد الغسل بواسطة NaOCl , EDTA و MTAD. **المواد وطرائق العمل:** ٣٦ضاحك سفلي استخدم في هذه الدراسة.تحضير العينات تم بواسطة مبارد البروتيبر إلى الحجم F3. العينات وزعت إلى ثلاث مجاميع تجريبية كل مجموعة احتوت عشر عينات كما يلي: المجموعة الأولى- العينات غسلت في النهاية لمدة خمسة دقائق في خمسة ملليلتر من ٥.٢٥ % NaOCl . المجموعة الثانية- العينات غسلت في النهاية لمدة خمسة دقائق في خمسة ملليلتر من ١٧% EDTA. المجموعة الثالثة- العينات غسلت في النهاية لمدة خمسة دقائق في خمسة ملليلتر من MTAD. ست عينات استخدمت كمجاميع سيطرة موجبة و سالبة, كل مجموعة احتوت ثلاث عينات. العينات حشيت بواسطة طريقة القمع الوحيد ما عدا العينات لمجموعة السيطرة الموجبة, والتي لم يتم حشوها. كل العينات حفظت بدرجة حرارة٣٧ مئوية لمدة سبعة أيام وبرطوبة مقدارها ١٠٠%. طريقة تغلغل الصبغة استخدمت لتقييم التسرب القمي. في النهاية البيانات جمعت وحللت احصائيا. **النتائج:** مجموعة ال MTAD أظهرت اقل تسرب متبوعة بمجموعة ال EDTA و مجموعة ال NaOCl أظهرت أعلى تسرب. مجموعة ال MTAD لم تختلف معنويا مع مجموعة ال EDTA , بينما مجموعة NaOCl اختلفت معنويا مع مجموعتي ال MTAD و ال EDTA. مجموعة السيطرة الموجبة بينت تسرب كامل للصبغة على طول الجذر بينما مجموعة السيطرة السالبة لم تبين اي تسرب للصبغة. **الاستنتاجات:** تحت ظروف هذه الدراسة, الغسل النهائي بواسطة MTAD أو EDTA افضل من الغسل النهائي بواسطة ٥.٢٥ % NaOCl فيما يخص أقل تسرب مجهري قمي.

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INTRODUCTION

Chemo mechanical preparation of root canals is the important stage for succeeding endodontic treatment [1]. Root canals instrumentation with manual or mechanical instruments generates smear layer on the walls of root canals [2]. The smear layer is granular, irregular, and amorphous layer attached to the surfaces of root canals [3]. It contains dental inorganic particles, and organic materials, which include residues of necrotic or vital pulpal tissue, and microorganisms with their by-products [4].

Apical seal capacity of the endodontic filling materials is the important requirement for proper root canal treatment [5]. Removal of smear layer via irrigation materials can augment adhesion of sealer to root canal walls. Consequently, the apical sealing capacity of endodontic filling materials is increased [6]. This is elucidated by that smear layer removal permits penetration of sealer into the dentinal tubules, [7] and both apical sealing capacity and bond strength increase [8].

Several endodontic irrigating solutions are utilized for eliminating smear layer [5]. Sodium hypochlorite (NaOCl) is more public solution, which is used during root canal treatment. It has antibacterial action and excellent activity for dissolving the organic tissue [9]. NaOCl is an alkaline solution with pH of 11 to 12 [10]. It can be used in concentrations of 0.5 % to 5.25 % during endodontic therapy [6].

Ethylene diamine tetra acetic acid (EDTA) is a chelation solution, which is broadly utilized for the removal of the smear layer [11]. Its principal activity is the removal of the inorganic part of smear layer [12]. It is available in paste and liquid forms with concentrations of 15%, and 17% [13]. During endodontic treatment washing with EDTA, and NaOCl is commended to eliminate inorganic and organic parts of smear layer [14]. Another Irrigation solution for smear layer removal is called MTAD. It consists of 4.25% citric acid, 3% doxycycline, and tween 80 (detergent) [15]. Many investigational studies revealed good results of MTAD in removing smear layer [3, 16].

The current study aims to assess apical micro leakage of root canals after final irrigation with NaOCl, EDTA, and MTAD by using the dye penetration method. The research hypothesis was that there would be no difference in the apical micro leakage after the final irrigation with different solutions which include NaOCl, EDTA, and MTAD.

MATERIALS AND METHODS

The existing study was accepted by a committee of research ethics at the dentistry college, Mosul University under number (UoM.Dent/ H.L.8/ 20).

36 mandibular premolars were collected and kept in distilled water. A radiograph was done for every tooth to eliminate the teeth with more than one root canal, twisted

canal, blocked canal, flared apex, and resorption defect.

Teeth crowns were transected by a diamond disk with water cooling at the level of the cervical line. Barbed broach (Densply Maillefer, Switzerland) was utilized for the extirpation of the pulp. Size 10 K-file (Densply Maillefer, Switzerland) was entered into canal of root till its tip was seen at the foramen apically. One millimeter was removed from the previous length for determination of working length.

All canals were instrumented by rotary ProTaper universal files (Densply Maillefer, Switzerland) to F3 size according to manufacturer instructions. ProTaper Universal rotary files were used with rotary endodontic handpiece (NSK, Japan); the rotation speed was 250 rpm, and torque 4 Ncm with gear reduction of 16:1. Each ProTaper file was discarded after the preparation of 4 samples. During instrumentation, all the samples were irrigated with 2 ml of 2.5% NaOCl (Clorox, Saso, Saudi Arabia) before the start of instrumentation and after each use of the file. The whole samples number was 36, these samples were distributed arbitrarily into 3 experimenting groups every group involved 10 samples and two control groups (positive and negative) every control group involved 3 samples as follows:

Group 1 – Samples were finally washed for 5 minutes with 5 ml of 5.25% NaOCl (1 ml / 1 minute).

Group 2 – Samples were finally washed for 5 minutes with 5 ml of 17% EDTA (1 ml / 1 minute).

Group 3 – Samples were finally washed for 5 minutes with 5 ml of MTAD (1 ml / 1 minute).

Positive control group – This group contained 3 samples. The first one was finally washed for 5 minutes with 5 ml of 5.25% NaOCl (1 ml / 1 minute), the second one was finally washed for 5 minutes with 5 ml of 17% EDTA (1 ml / 1 minute), and the third sample was finally washed for 5 minutes with 5 ml of MTAD (1 ml / 1 minute). All samples of this group were not obturated.

Negative control group – This group contained 3 samples. The first one was finally washed for 5 minutes with 5 ml of 5.25% NaOCl (1 ml / 1 minute), the second one was finally washed for 5 minutes with 5 ml of 17% EDTA (1 ml / 1 minute), and the third sample was finally washed for 5 minutes with 5 ml of MTAD (1 ml / 1 minute). All samples of this group were obturated as other samples of the experimenting groups.

After that, all the canals of the experimenting groups, and negative control group were dried by proTaper paper point (Densply Maillefer, Switzerland) size F3 and tug-back of size F3 ProTaper gutta-percha cone (Densply Maillefer, Switzerland) was tested. Then obturation of root canals with single cone technique was done by size F3 ProTaper gutta-percha

cone, and AD seal epoxy resin-based sealer (META BIOMED, Korea).

After Obturation, all the samples were sealed coronally with composan ceram composite resin (Promedica dental material GmbH, Germany), and were incubated for 7 days in 100% humidity at 37 ^{O}C to permit the whole sealer setting [17]. For assessment of apical leakage, all the samples of experimental groups were covered by two coatings of nail polish on the outer surfaces of the roots excepting the areas of apical 2 mm, which were not coated by nail polish [18, 19]. While, all the negative control samples were completely covered by two coatings of nail polish, and all the positive control samples were not coated by nail polish [19]. All samples then were dipped into 2% Methylene blue dye (Gurr®, BDH Chemicals LTD, England) for 3 days at 37°C [9, 17]. Subsequently, all samples were splashed by water and dried. Later, the nail polish coatings were eliminated by hand curette.

After that each root was fixed in the centre of mold which was made from silicone impression material and had dimension (3 cm length, 2.5 cm width, and 1.5 cm thickness) then EpoFix resin (Struers, Denmark) was poured inside the mold until the mold was filled. After 24 hours epoxy resin set was completed and a block of epoxy resin that contained root in its centre was ready for sectioning. Each block was sectioned longitudinally into slices of 1 mm by using Minitoms (Struers, Copenhagen, Denmark). Finally, the slices of each sample were observed under Stereomicroscope at 20X magnification. The apical leakage was measured from the apex of the root to the deepest extension of dye diffusion in millimetre. Slice that exhibited the highest apical leakage of each sample was recorded, while other slices of the same sample were neglected. After that data was collected, and statistically analyzed with SPSS Program version 21 for Windows software (IBM, USA). Statistical approaches that were used to analyze data included Descriptive Statistics, One Way Analysis of Variance (ANOVA), and Post Hoc Tukey Test.

RESULTS

Number of samples, mean, standard deviation, minimum and maximum values of apical dye leakage for experimental groups are listed in table (1). NaOCl group revealed the greatest mean of apical dye leakage (Figure 1) followed by EDTA group (Figure 2) while MTAD group revealed the least mean of apical dye leakage (Figure 3). There were significant differences among experimenting groups since p-value < 0.05 (Table 2). Post Hoc Tukey Test showed that MTAD group did not differ significantly with EDTA group, while NaOCl group differed significantly with MTAD and EDTA groups (Table 3). All positive control samples revealed dye diffusion through the whole root length (Figure 4), and this displays that the dye penetration technique was properly accomplished. While, all negative control

samples did not reveal any dye diffusion, and this illustrates that two coatings of nail polish were effective to prevent dye diffusion (Figure 5).

Table (1): Descriptive statistics for experimenting groups.

Irrigation	*N.*	*M.*	*St. D.*	*Mini.*	*Maxi.*
NaOCl	10	5.391	1.01719	4.07	6.53
EDTA	10	2.595	0.34086	1.98	2.90
MTAD	10	2.336	0.56398	1.32	2.87

M. = Mean, N. = Samples number, St. D. = Standard Deviation, Mini. = Minimum value, Maxi. = Maximum value.

Table (2): One Way ANOVA.

Source of variation	*sum of square*	*df*	*mean square*	*F*	*p*
Between groups	57.392	2	28.696	58.606	0.000**
Within groups	13.220	27	0.490		
Total	70.613	29			

** $p \leq 0.05$ means significant difference among experimental groups.

Table (3) Post Hoc Tukey Test**.**

Irrigation	*N.*	*M.*	*Tukey* +
MTAD	10	2.336	A
EDTA	10	2.595	A
NaOCl	10	5.391	B

+ Groups with different letters display significant difference between them, while groups with similar letter display non-significant difference between them.

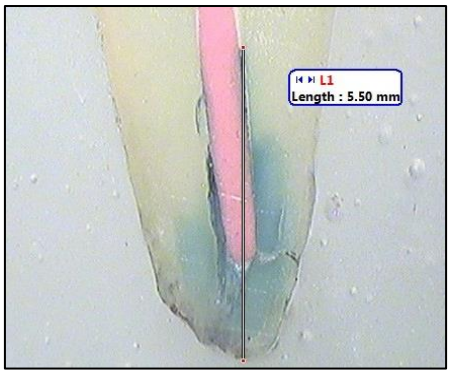

Figure (1): Stereomicroscopic picture of sample for NaOCl group (20X magnifying)

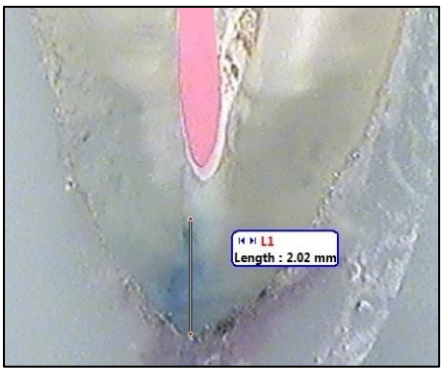

Figure (2): Stereomicroscopic picture of sample for EDTA group (20X magnifying)

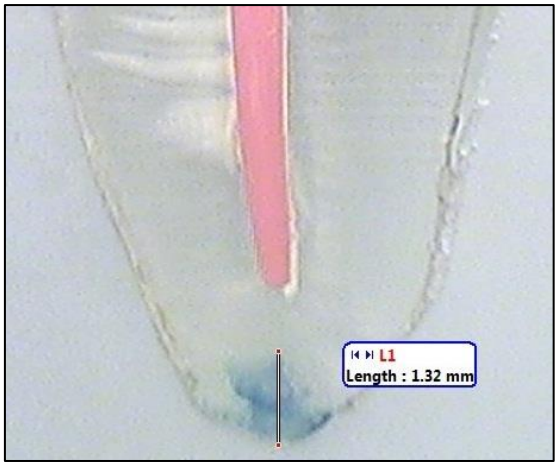

Figure (3): Stereomicroscopic picture of sample for MTAD group (20X magnifying)

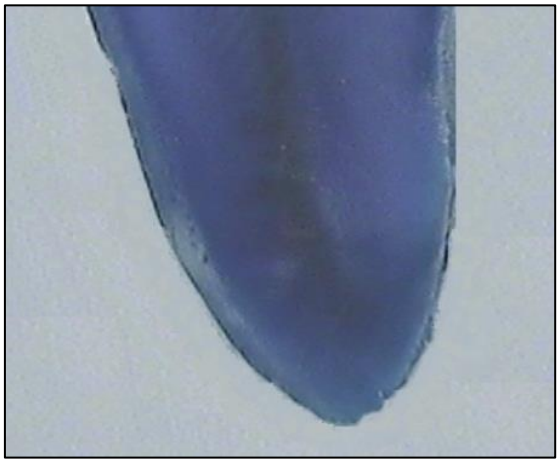

Figure (4): Stereomicroscopic picture of sample for positive control group (20X magnifying)

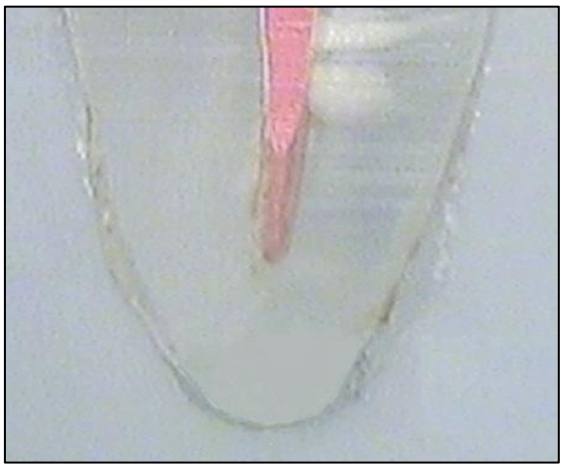

Figure (5): Stereomicroscopic picture of sample for negative control group (20X magnifying)

DISCUSSION

The whole seal of the root canals is a key for successful endodontic treatment [17]. Apical leakage is regarded the main reason for failure of root canal therapy. It is affected by numerous elements; one of them is the smear layer absence or presence [20]**.** Removal of smear layer via irrigating solutions increases adhesion of sealer to root canal wall subsequently, sealing capability is increased [6]. So, in this study, the apical leakage after irrigation with different solutions (NaOCl, EDTA, and MTAD) was evaluated.

Several methodologies are available for evaluating apical leakage one of them is dye penetration [21]. Method of dye penetration was used in the existing study for assessing apical leakage because it is easy to use, convenient, low cost, and sensitive technique [22]. Methylene blue dye was used in the existing study since it has a good ability for staining; in addition to that its molecular weight is lesser than those for microbial toxins. When the tiny molecules (dyes) can be prevented from penetration, bigger molecules (microbes and their by-products) are prevented from penetration too. Thus, the results of the methylene blue dye penetration method should be considered as a pointer for leakage possibility [23].

The least apical dye leakage of MTAD group can be explained by high MTAD capability to eliminate smear layer, and this can be ascribed to the synergistic activity of doxycycline, citric acid, and Tween 80 (detergent) which are existing in MTAD [24]. Citric acid is a chelating factor [24], demineralizes root canal surface. Subsequently, helps in the removal of smear layer [25]. Also, the existence of detergent (Tween 80) helps in reducing the surface tension for the root canal irrigation solution. Consequently, it improves the diffusion capability, and flow of MTAD solution deepest into the dentinal tubules [24]. So, the intimate contact of MTAD with the dentin surfaces of a root canal is enhanced even in the apical part of the canal [26].

Once MTAD is utilized as a root canal irrigation solution, smear layer "that is considered as a path for leakage [27]" is effectively eliminated [24] with definite demineralization of dentin surfaces [28]. This leads to better diffusion of sealer into the tubules of dentin [24]. Accordingly, mechanical locking of sealer with tubules occurs, and adhesion area between endodontic filling materials and walls of the root canal is increased [29]. Thus, apical leakage is reduced [30].

The low apical leakage of the EDTA group in the current study can be ascribed to the EDTA's ability to eliminate the smear layer that is related to its chelation action [24, 31]. Andrabi *et al*., [32] indicated that the actions of EDTA and MTAD to eliminate smear layer in the middle and coronal thirds were not different, while in the apical third smear layer removal by MTAD was better than that by EDTA. The less effectiveness for EDTA in the apical part can be ascribed to incomplete penetration of EDTA in apical zone [24, 31], and this is related to the high surface tension of EDTA [33]. Thus, non-significant more apical

leakage of EDTA group as a compare with MTAD group may be clarified. The absence of significant difference between EDTA and MTAD can be explained by that both EDTA and MTAD produced nearly complete removal of smear layer [26].

NaOCl is the more popular irrigating solution presently used because of its antimicrobial activity, and tissue dissolving ability. Furthermore, NaOCl is a potent lubricant [34]. On the other hand, the irrigation with NaOCl alone is not effective [32]. Scanning electron microscopic study that was achieved by Vallabahaneni *et al*., [35] revealed that samples, which were irrigated by 5.25% NaOCl displayed smear layer existence in all root parts, and this demonstrates the disability of NaOCl to eliminate smear layer. Accordingly, the highest significant apical leakage of NaOCl group in the current study can be explained.

Comparable results were reported by Balasubramanian *et al*., [5] who found that samples that were irrigated with MTAD showed better apical seal than those which were irrigated with EDTA. However, the difference between the two groups was not significant.

Hassan *et al*., [36] investigated apical bacterial leakage of root canals after washing by MTAD, and NaOCl. They indicated a significant increase in apical bacterial leakage of the samples, which were irrigated with NaOCL than those, which were irrigated with MTAD in all periods, and these results are in accordance with the results of the present study.

Andrabi *et al*., [32] concluded that NaOCl failed to remove the smear layer, and the difference was non-significant between MTAD, and EDTA groups at the middle, and coronal parts, but at apical part, MTAD was better in elimination of smear layer. Nogo-Živanovi *et al*., [3] reported that EDTA group did not differ significantly with MTAD group in the middle, and coronal parts of root canals, but in the apical part MTAD group was significantly better than EDTA group in elimination of smear layer. Consequently, the results of these studies can be considered in agreement with the results of the existing study.

CONCLUSIONS

Under the conditions of the existing study, it was concluded that the final irrigation with either MTAD or EDTA is better than final irrigation with 5.25 % NaOCl in terms of less apical microleakage of the obturated root canals.

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