THE INFLUENCE OF PREPARATION SIZE ON EFFICACY OF EDTA FOR SMEAR LAYER REMOVAL IN THE APICAL PART OF ROOT CANAL: AN IN VITRO SEM STUDY

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ABSTRACT

The influence of preparation size on efficacy of EDTA for smear layer removal in the apical part of root canal: an in vitro SEM study

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Background: Success of RCT relies on proper chemomechanical preparation, effective disinfection of root canal system and its three-dimensional obturation. During canal preparation a smear layer cover is produced. Adequate removal of this smear layer using EDTA is essential for the success of infected root canal treatment. Several parameters, including apical size preparation, affects the smear layer removal ability.

Aim: To study the role of the size of apical root canal preparation on removal of smear layer after irrigation with EDTA solution on extracted human teeth.

Materials and Methods: Forty-two extracted mandibular molars were included in the study. Radiographic examination with two views, mesio-distal and bucco-lingual, were taken for examination of distal canal. Teeth were selected according to specific inclusion criteria. They were divided into three groups; each contains fourteen samples. Working length was set at minor apical foramen, 1mm from major apical foramen visible under dental operating microscope. Protaper universal system was used for distal canal preparation with the Master Apical File as follows: Group A finishing at F2 (#25/0.08), Group B finishing at F4 (#40/0.06) and Group C finishing at F5 (#50/0.06). Full irrigation protocol was followed with 2ml of sodium hypochlorite in a concentration 5.25%, used between each file and 3ml after completion of canal preparation with activation 20 seconds for 3 cycles using Endo activator. 2ml EDTA solution in a concentration 17% was used for 1 minute with activation using Endo Activator.

A 30-guage side vented needle was used for irrigation and set 1mm from minor apical foramen. Final irrigation was done with 5ml saline.

Specimens were decoronated and distal root was sectioned bucco-lingually into two halves. The half which showed better apical configuration was selected and immersed in alcohol (70%) for 20 minutes for dehydration. The specimens were further sputter-coated with carbon (C) under vacuum and examined in a Scanning Electron Microscope. The SEM analysis was done at the General Department of Forensic Science and Criminology at the Dubai Police Head Quarter. SEM images were taken at three levels of the apical end, at 2mm, 4mm and 6mm. Total of 6 images fro each specimen. Images were randomly arranged for smear layer evaluation. The evaluation was performed by two blinded observers (endodontists) and re-evaluation was done at a second session with 30 days interval. Images evaluation was done on the basis of a 4-grade score system as has been proposed by Hulsmann et al. 1997.

Results: Clear root canal walls were found throughout the apical half of the roots in only 11.9%, 19 % and 54.7% of the samples in groups A, B and C, respectively. In comparison between groups, Group C showed to have significantly better scores ($p=0.000$) than other groups with 81% of the samples had less than 50% of the dentinal walls free of smear layer/debris, while it was only 45.2% and 35.7% of the samples in group B and A respectively. In comparison between levels, at level of 2mm, group C had significantly better results in estimating 0-50% presence of smear layer/debris in the scanned dentinal walls areas ($p=0.041$). At level of 4mm, also group C showed significantly better results in estimating 0-50% presence of smear layer/debris in the analyzed dentinal walls areas ($p=0.002$) and in estimating 0-15% of the presence of smear layer analysis ($p=0.004$). At level of 6mm, significantly better scores in estimating 0-15% of the presence of smear layer/debris in the scanned dentinal walls areas was found in group C ($p=0.023$).

Conclusion: Significant differences between the groups of teeth with different size of apical enlargement were found when various measurement criteria were performed. The present

results clearly indicate that the ability of irrigation to remove smear layer/debris from the instrumented straight root canals at their apical part is enhanced with increasing the size of apical preparation with the Protaper rotary system.

DEDICATION

I would like to dedicate my thesis and my work to my lovely family, who supported me during my journey in HBMCDM. Also, I dedicate my thesis to Al Jalila Foundation for their scholarship and for providing me this lifetime opportunity, without them my dream of endodontic specialty would not come true.

DECLARATION

I declare that all the content of this thesis is my own work. There is no conflict of interest with any other entity or organization

Name: Reem Juma Obaid Juma AlMutawa

Signature:

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1. INTRODUCTION

1.1. Objective of root canal treatment

The main objective of root canal treatment (RCT) is to treat or prevent apical periodontitis [1, 2, 3]. Success of RCT relies on proper chemo-mechanical preparation, effective disinfection of the root canal system and its three-dimensional obturation [1, 2]. Instrumentation of root canals represents an essential step in shaping and cleaning of the root canal system, which further facilitates its disinfection by means of various irrigation solutions and intracanal medicaments [1,2,3].

Preparation of the root canal system is one of the most important stages in root canal treatment, including removal of vital and necrotic tissue with infected or non-infected root dentine. It aims to clean and shape the root canal system to facilitate the cleaning of root canal walls, the refreshment of irrigants to more effectively eradicate bacteria and eliminate bacterial byproducts, the placement of intracanal medicaments for complete disinfection control and the 3D obturation. Mechanical root canal preparation has anatomical and microbiological challenges. Anatomical challenges including wide variations in the number, length, curvature and diameter of root canals; the complexity of the apical anatomy with accessory canals and ramifications; communications between the canal space and the lateral periodontium and the furcation area and the anatomy of the peripheral root dentine. Microbiological challenges as both pulp tissue and root dentine may harbor microorganisms and toxins [4].

1.2. Chemomechanical preparation of root canal

Chemomechanical preparation of the root canal through a combination of mechanical instrumentation and antibacterial irrigation is a critical stage in canal disinfection. This is followed by placement of a root canal filling and coronal restoration in order to seal potential avenues of entry of micro-organisms into the root canal, and to entomb any remaining microorganisms in order to prevent their proliferation [5].

During mechanical instrumentation of root canals, with hand or rotary instruments, an amorphous irregular and granular layer (smear layer) which contains inorganic and organic material from pulp tissue, bacteria and their by-product is created. The thickness of smear layer may depend on the type of preparation technique, mechanical properties of the cutting instruments and the amount of water in the dentine when it had been cut. The smear layer covers irregularly the prepared dentinal walls along the entire root canal system. It can be detected using scanning electron microscope (SEM) and reported for the first time by Eick et al. [6]. It has been well characterized that the presence of smear layer affects the successful outcome of RCT, as it contains bacteria and their by-products and it blocks the dentinal tubules and extends superficially into them, preventing penetration of the antibacterial irrigants and medicaments. In addition to that, it influences the penetration of filling materials to the canal walls and increases the apical microleakage after obturation. For all these mentioned reasons, root canal cleaning and shaping should also focus on removal of the smear layer to enhance successful root canal outcome [7].

1.2.1. Irrigants used in root canal treatment

The chelating agents such as Ethylenediaminetetraacetic acid (EDTA) are used specifically to remove the smear layer formed during canal preparation, but their ability to reach and remove the formed layer may be affected by insufficiently prepared canals. Therefore, the clinician must enlarge the canal space to a sufficient degree to allow adequate chemomechanical instrumentation into all areas of the root canal system, including the apical third [8].

In today practice the irrigation with sodium hypochlorite and EDTA has been accepted as the most effective method of organic material dissolution, smear layer removal and disinfection in the instrumented root canal system. Previous studies have investigated in vitro tooth models showed the efficacy of EDTA for smear layer removal in the coronal and middle thirds of the root canals [1]. However numerous studies demonstrated that irrigation with EDTA is less

effective in the apical part of the canal due to narrow root canal space and limited irrigant flow space and refreshment [2, 9].

EDTA is a chelating agent that dissolves the smear layer and increases the dentin permeability. It was first introduced to aid in the root canal treatment of calcified and narrow canals. It is a complex molecule that binds to divalent and trivalent metal ions such as calcium forming a stable complex and no further dissolution takes place. The properties of EDTA is self-limiting due to pH changes that occur during the demineralization process. The effective exposure time of EDTA (17%) on the dentin surface is 1 minute as more exposure could lead to dentinal softening and recent studies had showed that no difference in the amount of extracted calcium. In addition, EDTA efficacy depends on the available root canal space, as in narrow canals insufficient amount can be introduced leading to insufficient removal of smear layer [10].

Beside EDTA, sodium hypochlorite solution (NaOCL) is used as the standard irrigant during chemomechanical preparation of the root canal system. It was introduced as the main irrigant in Endodontics in the early 1920. It has a broad-spectrum antimicrobial efficacy beside its necrotic tissue dissolving ability [11]. NaOCl ionizes in water into sodium (Na+) and the hypochlorite ions, OCl–, and establishes an equilibrium with hypochlorous acid (HOCl). It is used in concentrations between 0.5-6% and in order to keep it active, continuous irrigation or agitation is required. It should be used between each file and for 1 to 2 minutes after instrumentation [2].

1.2.2. Role of apical preparation size

It has been suggested that apical enlargement during root canal preparation plays an important role in achieving the goal of proper smear layer removal from the apical root canal [12]. The apical width plays an important role in root canal treatment outcome [2]. The larger the apical preparation, the greater the flow of irrigant solution and greater the reduction in intracanal bacteria. It is explained that as a larger size file used in the canal, it has better contact area with

the canal walls which may lead to improved removal of infected dentin and adherent smear layer and higher chance of eliminating canal irregularities. In addition, it allows deeper penetration of the irrigation needle allowing more chemical effect of the solutions on the canal reaching to deeper apical parts. The greater the amount of irrigants used, the better bacterial reduction leading to improved outcome of root canal treatment [13].

Weine [14] defined the master apical file as enlarging the apical portion of the root canal system three sizes larger than the first binding file. However, several morphological studies during the last 5 decades have shown the irregularities of apical anatomy in shape. Since the oval shape of the apical part of the canal is the most common configuration, the binding file cannot reflect the true canal diameter [15]. It seems reasonable to suggest that the rule to finishing the canal preparation three sizes larger than the first binding file does not insure complete instrumentation of the apical dentin. Furthermore, the limited space provided by this preparation size may also affects the efficacy of irrigation solutions in root canal smear layer removal and disinfection.

2. REVIEW OF THE LITERATURE

So far, data from clinical and experimental in vitro studies are controversial:

In 1991, a comparison was designed between different sizes of enlargement of apical part of the canal with the use of calcium hydroxide as intracanal medicament, but the results showed insignificant difference between enlarged group and control group [16].

In 1994 Yared and Dagher compared canal preparation size #25 and #40 and the results showed there was no significant difference in bacterial reduction between the two sizes [17].

Coldero et al. [18] reported reduction in intracanal bacteria during root canal preparation with and without apical enlargement. The result showed that there was no significant difference in intracanal bacterial reduction when Nickel–Titanium GT rotary preparation with NaOCl and EDTA irrigation was used with or without apical enlargement preparation technique.

Also, in the same year, Card et al. [19] investigated whether instrumentation to sizes larger than typically used would more effectively remove cultural bacteria from the canals of canines, premolars and molars. The results showed simple root canal system without communication may be rendered bacteria free with larger apical preparation.

In 2005 Hulsmann [20] concluded that the desirable final size of apical preparation remains controversial. Several comparative investigations of pre and postoperative cross-sections of mesio-buccal root canals in curved mandibular molars resulted in 3 to 18 out of 25 specimens with more than 25% of the diameter left unprepared following preparation with different rotary NiTi systems to size #45.

Khademi et al. [21] conducted a study to determine the minimum instrumentation size required for the effective penetration of irrigants and elimination of debris and smear layer from the apical third of the root canals. Mesiobuccal canals of 40 extracted human mandibular first molar teeth were instrumented using crown-down technique and divided into 4 experimental groups with apical preparation size #20, #25, #30 and #35. The samples were sectioned and reviewed under scanning electron microscope. Results showed that the minimum preparation

size required for irrigant penetration to the apical third of the canal is size #30. Furthermore, a histological evaluation of the effectiveness of increased apical enlargement for cleaning the apical third of curved canals compared the amount of dentinal debris between files size #30, #35 and #40, #45. Authors reported significant reduction in the remaining debris when larger file sizes used [22]. Tabrizizadeha and Shareghib [23] used scanning electron microscope, to compare the effect of smear layer removal in two different groups using two apical preparation sizes. They concluded that increasing the canal preparation size did not lead to better cleanliness of the canal walls or to a more efficient smear layer removal. The irrigant penetration into the apical canal and removal of debris is dependent on the final size of the instrument used in the canals [24]. Rodrigues et al. [25] showed that the larger the apical preparation size of infected root canals, the greater the intracanal bacterial reduction. In the randomized control trial of Saini et al. [26], the results showed a statistically significant reduction in the periapical index score and increase in the proportion of successfully healed teeth in patient with increased apical preparation of the root canal. The most recent study conducted in 2019 by Butcher et al [8], it is a SEM study investigated the relationship between final apical preparation size and smear layer removal in the apical third using conventional irrigation in mandibular bicuspids. Samples were divided into five groups and instrumented up to size 25, 30, 35, 40, or 45 with 0.04 taper using 2.5% sodium hypochlorite (NaOCl) as an irrigant. Final irrigation was performed with 17% EDTA followed by 2.5% NaOCl. They concluded that apical enlargement more than size 35/.04 is essential to enhance removal of smear layer at the apical third.

Among previous studies there is only one randomized controlled clinical trial which indicated that patients with necrotic pulp undergoing root canal treatment with increased apical enlargement had better healing outcome than the group with smaller apical diameter. A systematic review further showed, that only few studies have addressed the clinical outcome of various master apical file sizes and the ideal MAF remains a mystery in any individual canal.

It is clear that more evidence-based research is required as literature failed to show what an optimal apical preparation diameter might be [27].

In summary, two conflicting working hypotheses have been extensively discussed in the literature during the last 3 decades. The one working hypothesis states that the apical enlargement plays an important role in providing adequate penetration depth for the irrigant resulting in better cleaning effect. Supporting studies have shown that instrumentation leaves the canal with smear layer which reduces the penetration depth of irrigant, intracanal medicament, sealer and obturation material. The alternative hypothesis is based on the concerns of minimum invasive preparation techniques in endodontics and the risks of procedural errors (transportation or perforation) because of apical enlargement which weakens the root canal walls. In a number of studies, it has been reported that enlargement of the apical root canal system did not ensure removal of the inner layer of dentine from all apical root canal walls or all infected necrotic pulp tissue [28, 29]. Dalton et al. [30] and Shrikanth et al. [31] also showed that with increasing file size, there was an increased reduction in bacteria and increased risk of fracture.

3. AIM

Aim of this research was to study the role of the size of apical root canal preparation on removal of smear layer after irrigation with EDTA solution in extracted human teeth.

Objective of the study:

To compare the effects of EDTA irrigation on smear layer and debris removal efficacy in single canals, of the distal roots of lower molars when canals had been instrumented by Protaper Universal System up to size #25/.08, #40/.06, #50/.06, leading to the following cross-sectional diameters shown in table 1:

Working length in mm	25/.08 taper	40/.06 taper	50/.06 taper
1mm	0.33	0.46	0.56
2mm	0.41	0.52	0.62
3mm	0.49	0.58	0.68
4mm	0.57	0.46	0.74
5mm	0.65	0.70	0.80
6mm	0.73	0.76	0.86

Table 1. cross-sectional diameters of prepared canals.

Null hypotheses:

There are no differences among the surface structure and presence of smear layer/debris in the apical root dentin of extracted human teeth after instrumentation with the Protaper Universal System between the following MAF sizes:

- #25/.08 and #40/.06 (hypothesis 1).
- #25/.08 and #50/.06 (hypothesis 2).
- #40/.06 and #50/.06 (hypothesis 3).

4. MATERIALS AND METHODS

The present in vitro experimental study was approved by the Research and Ethics committee of Hamdan Bin Mohammed College of Dental Medicine, MBRU on meeting held on 15 October 2017.

Teeth selection

Forty-two freshly extracted human mandibular molars with straight distal roots were selected from various dental clinics and stored initially in 70% alcohol solution and then in normal saline throughout the study.

The inclusion criteria in clinical examination for sample selection were:

a. Mandibular molar teeth fully developed.
b. Teeth with intact straight roots.
c. Teeth without or only minimal coronal restorations.
d. Teeth with root length less than 18 mm.

Radiographic Examination: radiographs were taken in bucco-lingual and mesio-distal directions, in order to standardize the anatomical features of the root canals in the specimens and the typical internal tooth morphology (absence of intrapulpal calcifications and narrow canals). Only distal roots classified in the Vertucci type I group were used. Curved canals and other Vertucci classifications have been excluded.

Experimental procedures

Access Cavity: The typical regular trapezoidal access cavity for lower molars have been prepared to explore the pulp chamber. After preparation of the access cavity a size 10 stainless steel K file was inserted into the distal root canal until the file tip was visible at the major apical foramen by 25x magnification power of microscope (Leica M320, Germany). Apical end of working length was set at the minor apical foramen which was estimated at 1 mm less of the file length up to major apical foramen.

Instrumentation of the root canals: The following steps were followed for instrumentation of the distal root canals on selected teeth:

1. Initial preparation with glide path (Sx) and with apical patency by using K-file #10.
2. All distal roots were prepared with Protaper Universal rotary system 25 mm files according to the manufacturer's instructions and guidelines as follows: S1 (17/.02), S2(20/.04), F1 (20/.07) with rotary device, Protaper universal system, (DENTSPLAY MAILLEFER, Switzerland) and speed 300 RPM and torque 4 Ncm.
3. By using the same rotary device system of Ni-Ti files and under the same speed and torque, root canals were further prepared differently in 3 groups of 14 randomly divided teeth, in order to set the size of Master Apical Files as follows:

- Group A. Root canal instrumentation was finished with F2 (#25 /.08)
- Group B. Root canals were instrumented with F3 (#30/.09) and preparation was finished with F4 (#40 /.06)
- Group C. Root canals were instrumented with F3 (#30/.09), F4 (#40/.06) and preparation was finished with F5 (#50 /.06).

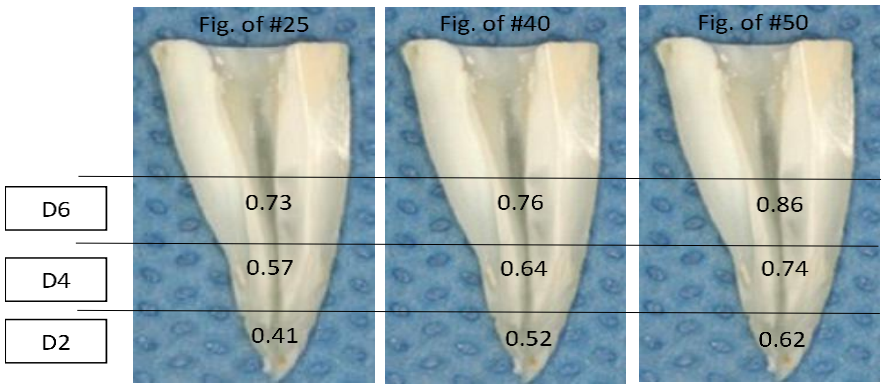

Figure 1 shows comparatively the profile of 6 apical mm of master apical files in the 3 groups of teeth.

Irrigation of the root canals: Full irrigation protocol applied for all distal root canals of the selected teeth by using 30-gauge side vented needles (DIA DENT, Korea). 2ml of sodium hypochlorite (5.25%) used after each file during instrumentation at a distance 1mm from the minor apical foramen. After completion of canal preparation to the full working length irrigation the following steps were used:

- Irrigation with 3ml sodium hypochlorite (5.25%) and activation for 3 cycles of 20 seconds using Endo Activator (DENTSPLAY MAILLEFER, Switzerland).
- Irrigation with 2ml saline.
- Irrigation with 2ml of 17% EDTA solution for one minute and agitation with Endo Activator.
- Irrigation with 5ml saline.
- Drying of the root canals with paper points.

Root sectioning, SEM examination and evaluation: Teeth were decoronated and the distal roots were further prepared for examination. Longitudinal lingual and buccal groves prepared by using a thin diamond needle bur at high speed and teeth were separated into two halves with orthodontic wire cutter. Then the specimens immersed in alcohol (70%) for 20 minutes for dehydration and the one of the halves showing better apical root canal configuration was used as coded specimen. The specimens were further sputter-coated with carbon (C) under vacuum and examined in a Scanning Electron Microscope (Quanta Feg 650, USA). SEM analysis was done at the General Department of Forensic Science and Criminology at Dubai Police Head Quarter. The dentinal surfaces of the apical part of root canals were examined by using a standardized magnification (500X) at three levels from the minor apical foramen, at 2mm, 4mm and 6mm. One representative area was selected at each level, from which two images at magnifications of 500X and 1000X were taken (total 6 images for each root canal).

Smear layer and agglomerations of debris analyzed on the basis of a 4-grade score system as has been proposed by Hulsmann et al. 1997 [32].

- Score 1: Agglomerations of debris and smear layer covering less than 15% of the dentinal tubules.
- Score 2: Agglomerations of debris and smear layer covering 15% to 50% of the dentinal tubules.
- Score 3: Agglomerations of debris and smear layer covering 50% to 80% of the dentinal tubules.
- Score 4: Complete root canal wall covered by a homogenous smear layer or agglomerations of debris with rare open dentinal tubules (over 80%).

Images of the coded specimens were further randomly arranged. Scoring procedure was performed by two blinded observers (endodontists) in two scoring sessions (30 days interval). Both intra-observer and inter-observer variations were estimated.

In the cases of intra-observer scoring differences a third evaluation was performed. In the case of inter-observer scoring differences the more severe score was used.

Statistical Analysis: Data was entered on computer using IBM-SPSS for Windows version 23.0 (SPSS Inc., Chicago, IL). Frequency tables, bar graph and measure of percentage were performed as descriptive. Categorical variables were cross-tabulated to examine the independency between variables, for such variables the $\chi 2$ -square test or Fisher's exact test as appropriate were used. A P value of less than 0.05 will be considered significant in all statistical analysis.

5. RESULTS

A. Smear layer/debris evaluation per group

The results of SEM analysis of the surface structure of root canal walls per each one group are as follows:

Group A -The results of SEM analysis of the surface structure of root canal walls of group A are seen in table 2 and Fig. 2. Presence of a heavy smear layer/debris was found throughout the apical half of the roots in 64.9 % of the samples.

Dentinal tubules were not visible in 50 % of the samples. Clear root canal walls (> than 85% of dentinal tubules open), were found in only 11.9 % of the samples. No significant difference was found between the 3 levels of smear layer/debris evaluation (table 2) in terms of 50% of the samples(p=0.113).

Group A	Score 1	Score 2	Score 3	Score 4
at 2mm	2	2	1	9
at 4mm	0	3	3	8
at 6mm	3	5	2	4
Total	5	10	6	21
%	11,90%	23,80%	14,20%	50%

Table 2. Smear layer/debris evaluation in group A

Figure 2A. SEM image of group A at the level of 2 mm (1000x).

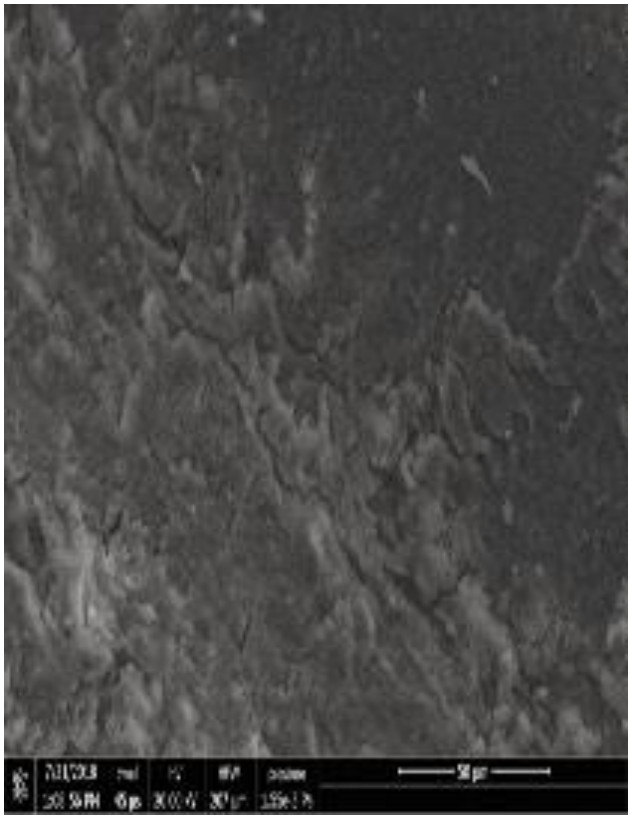

Figure 2B. SEM image of group A at the level of 4 mm (1000x).

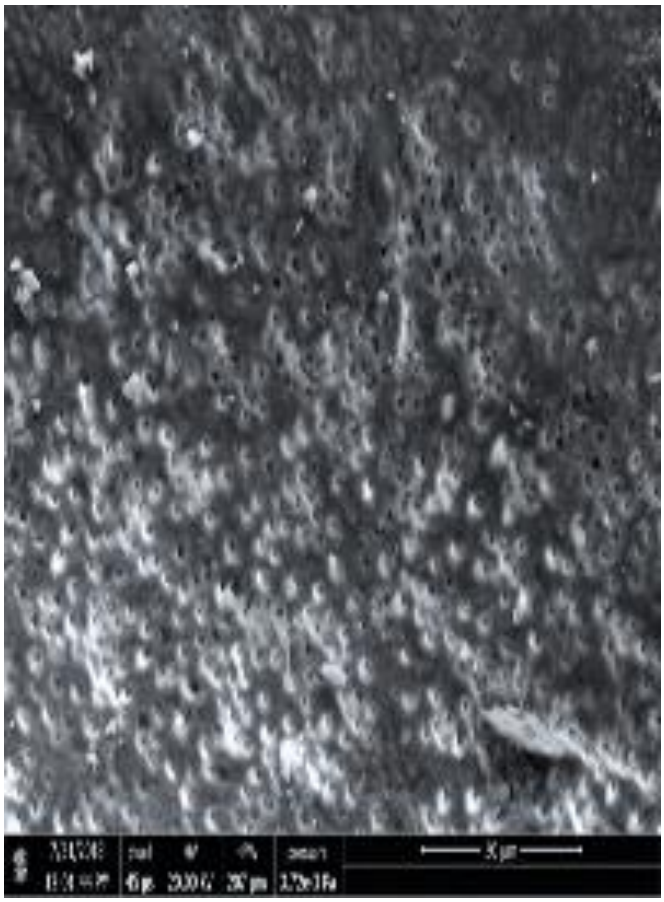

Figure 2C. SEM image of group A at the level of 6 mm (1000x).

Group B - The results of SEM analysis of the surface structure of root canal walls of group B are seen in table 3 and Fig. 3. Presence of a heavy smear layer/debris was found throughout the apical half of the roots in 54.7 % of the samples. Dentinal tubules were not visible in 33.3 % of the samples.

Clear root canal walls (> than 85% of dentinal tubules open), were found in only 19 % of the samples. Significantly better scores at the level of 6 mm from the apical end (table 3) in terms of 50% of the samples was found in group B (p=0.029)

Group B	Score 1	Score 2	Score 3	Score 4
at 2mm	1	2	5	6
at 4mm	2	4	3	5
at 6mm	5	5	1	3
Total	8	11	9	14
%	19%	26,10%	21,40%	33,3

Table 3. Smear layer/debris evaluation in group B

Figure 3A. SEM image of group B at the level of 2 mm (1000x).

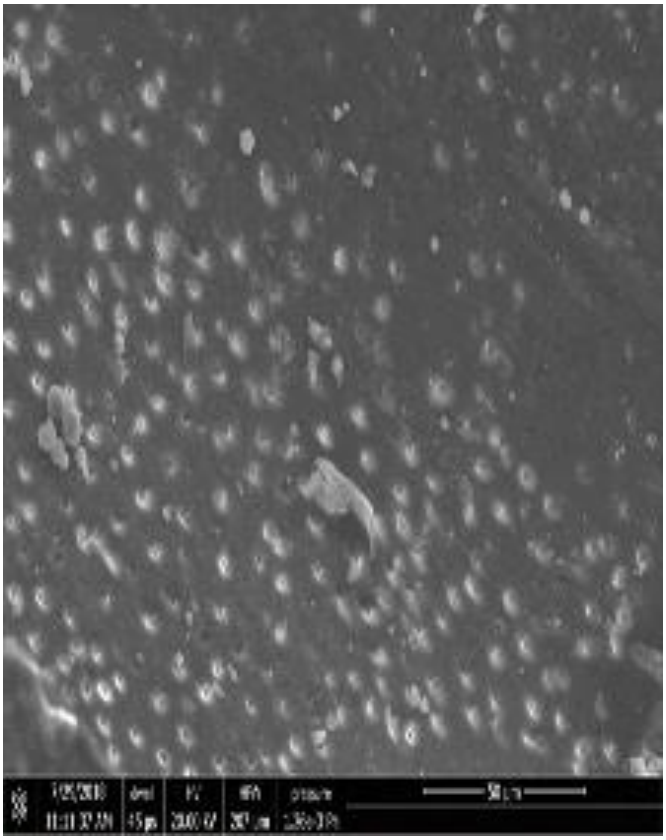

Figure 3B. SEM image of group B at the level of 4 mm (1000x).

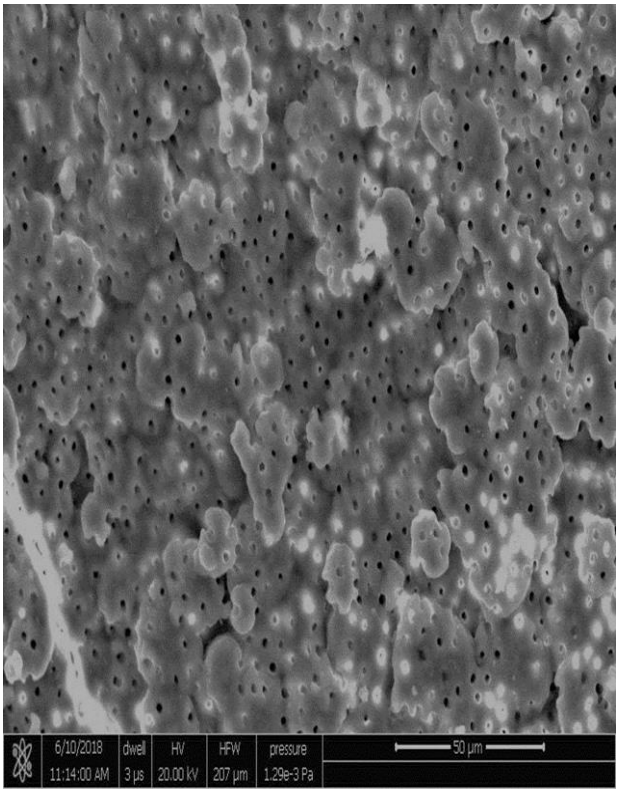

Figure 3C. SEM image of group B at the level of 6 mm (1000x).

Group C - The results of SEM analysis of the surface structure of root canal walls of group C are seen in table 4 and Fig.4. Presence of a heavy smear layer/debris was found throughout the apical half of the roots in 19% of the samples. Dentinal tubules were not visible in 7.1% of the samples.

Group C	Score 1	Score 2	Score 3	Score 4
at 2mm	6	4	3	1
at 4mm	7	5	0	2
at 6mm	10	2	2	0
Total	23	11	5	3
%	54,70%	26,10%	11,90%	7,10%

Table 4. Smear layer/debris evaluation in group C

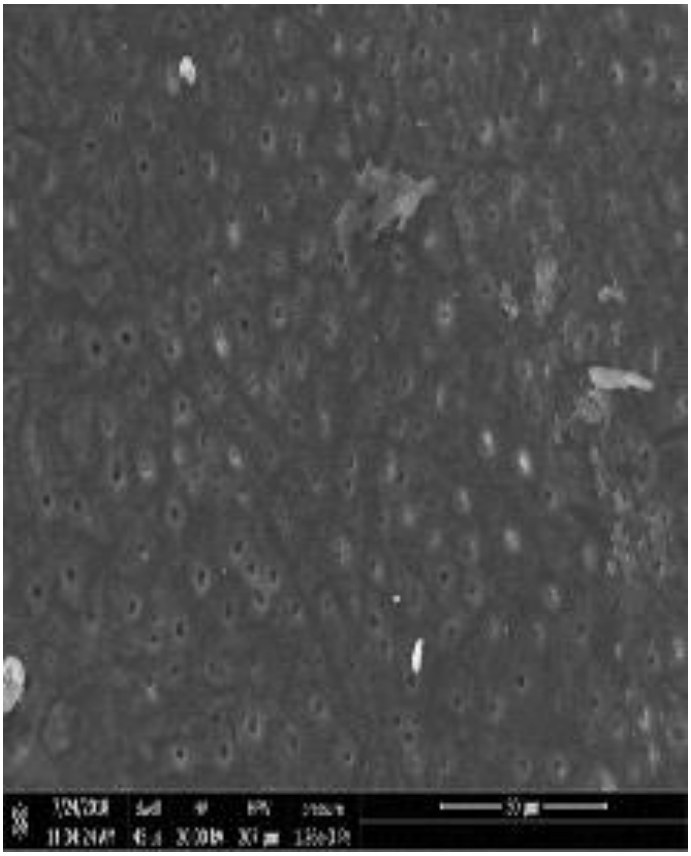

Figure 4A. SEM image of group C at the level of 2 mm (1000x).

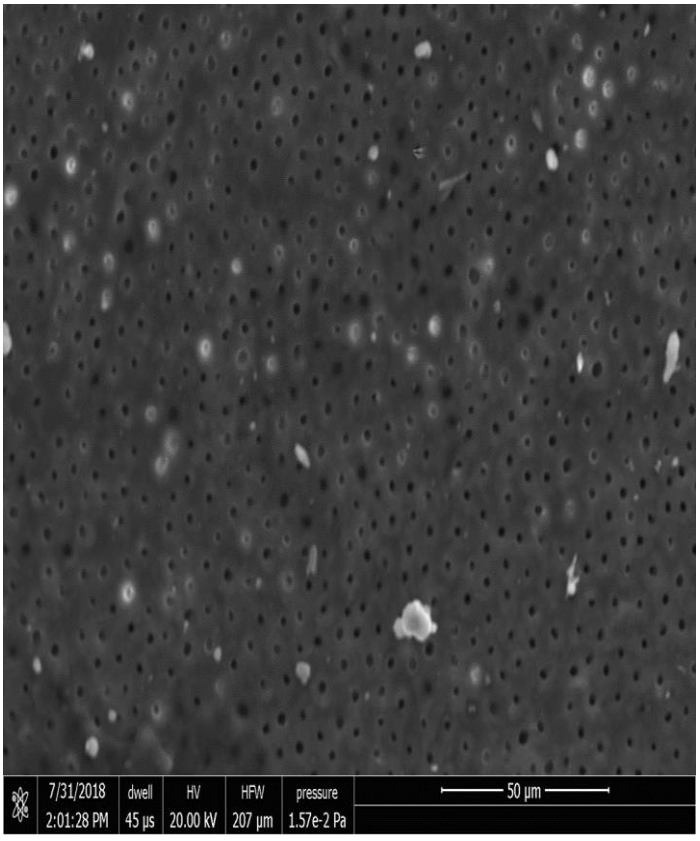

Figure 4B. SEM image of group C at the level of 4 mm (1000x).

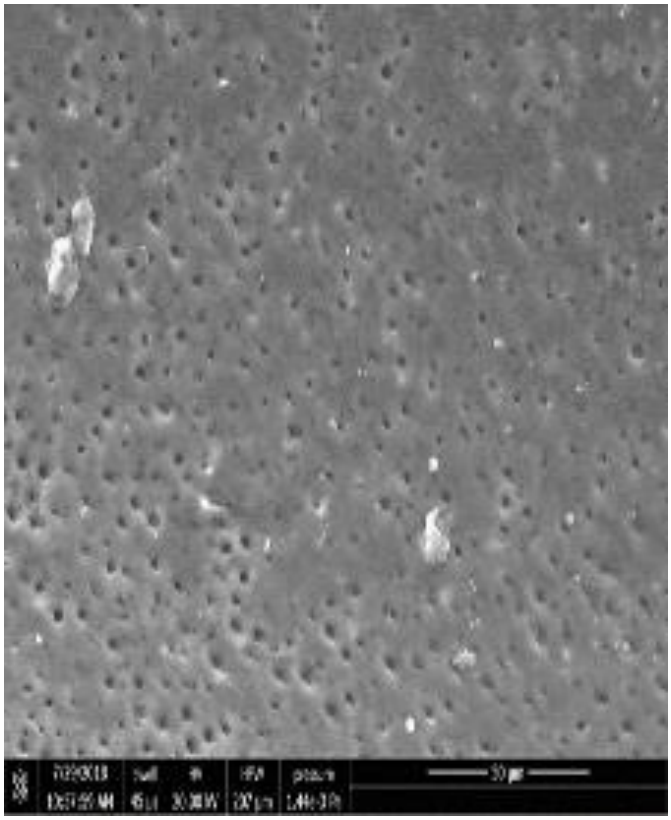

Figure 4C. SEM image of group C at the level of 6 mm (1000x).

Comparative results per group

Comparison of the results of SEM analysis of the surface structure of root canal walls between the three groups is seen in Fig. 5. It shows that group C had 81% of samples less than 50% of dentinal wall free form smear layer/debris, significantly better that the other groups (p=0.000). In table 5 the smear layer/debris scores in terms of 50 % of the samples can be seen comparatively.

Group	Group A		Group B		Group C	
	No (%)		No (%)		No (%)	
Result	< 50	>= 50	<50	>=50	<50	>=50
2 mm	4(28.6)	10 (71.4)	3(21.4)	11(78.6)	10(71.4)	4(28.6)
4 mm	3(21.4)	11(78.6)	6(42.9)	8(57.1)	12(85.7)	2(14.3)
6 mm	8(57.1)	6(42.9)	10(71.4)	4(28.6)	12(85.7)	2(14.3)
p value	0.113		0.029		0.539	

Table 5. Comparative results of evaluation in the three groups in estimating >50% or <50% of the presence of smear layer/debris in the samples.

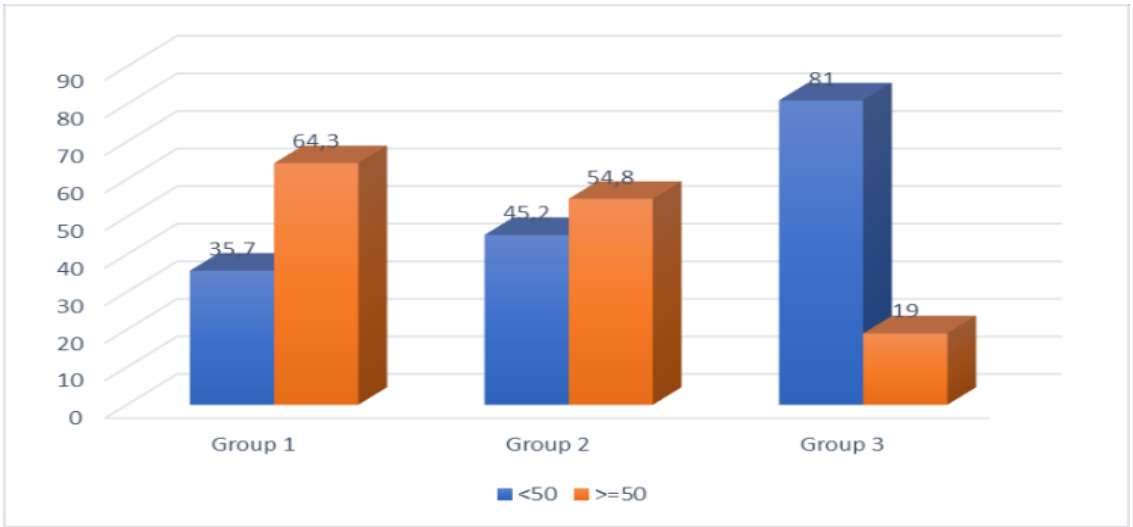

Figure5: Comparison of the smear layer evaluation between the 3 groups.

Comparative results per level of analysis

Level 2 mm

Comparative analysis per level was performed in estimating 50% (table 6) and 15 % (table 7) of the presence of smear layer/debris in the analyzed dentinal walls areas.

Significantly better scores at the level of 2 mm from the apical end (table 6) in estimating 50% of the presence of smear layer/debris in the analyzed dentinal walls areas was found in group C (p=0.041).

No significant difference was found at the level of 2 mm from the apical end between the three groups (p=0.247) in estimating 15% (table 7) of the presence of smear layer/debris in the analyzed dentinal walls areas (p=0.051).

Level 4 mm

Significantly better scores at the level of 4 mm from the apical end (table 6) in estimating 50% of the presence of smear layer/debris in the analyzed dentinal walls areas was found in group C (p=0.002)

Significantly better scores at the level of 4 mm from the apical end (table 7) in estimating 15% of the presence of smear layer/debris in the analyzed dentinal walls areas was found in group C (p=0.004).

Level 6 mm

No significant difference was found at the level of 6 mm from the apical end (table 6) between the three groups in estimating 50% of the presence of smear layer/debris in the analyzed dentinal walls areas (p=0.247). Significantly better scores at the level of 6 mm from the apical end (table 7) in estimating 15% of the presence of smear layer/debris in the analyzed dentinal walls areas was found in group C (p=0.023).

Level	%	Group A	Group B	Group C	p value
2 mm	< 50	4(28.6)	3(21.4)	10(71.4)	0.041
	>= 50	10 (71.4)	11(78.6)	4(28.6)	
4 mm	< 50	3(21.4)	6(42.9)	12(85.7)	0.002
	>= 50	11(78.6)	8(57.1)	2(14.3)	
6 mm	< 50	8(57.1)	10(71.4)	12(85.7)	0.247
	>= 50	6(42.9)	4(28.6)	2(14.3)	

Table 6. Comparative results of evaluation in the three groups in estimating >50% or <50% of the presence of smear layer/debris in the analysed dentinal walls areas per level of analysis.

Level	%	Group A	Group B	Group C	P value
2 mm	< 15	2(14.3)	1(7.2%)	6(42.8%)	0.051
	>15	12(85.7%)	13(92.8%)	8(57.2%)	
4 mm	< 15	0(0%)	2(14.3%)	7(50%)	0.004
	>15	14(100%)	12(85.7%)	7(50%)	
6 mm	< 15	3(21.5%)	5 (35.7%)	10 (71.4%)	0.023
	>15	11(78.5%)	9(64.3%)	4 (28.6%)	

Table 7. Comparative results of evaluation in the three groups in estimating >15% or <15% of the presence of smear layer/debris in the analysed dentinal walls areas per level of analysis.

6. DISCUSSION

Chemomechanical debridement of root canals is essential to reach a clinically satisfactory level for the success of endodontic therapy [1, 2, 3]. Root canal treatment procedures alter the canal surface depending upon the canal anatomy, the type and sequence of used instruments and the chemicals used to facilitate debridement. The canal preparation leaves behind smear layer which affects the success of root canal treatment [6]. The use of chelators, like EDTA, have been shown to dramatically increase the smear layer/debris -free surface of the prepared root canal walls [7]. However, it is not well known if the size of preparation affects the cleaning of the apical part of the root canals. It is reasonable to suggest that the narrower anatomy of the apical root end renders the area less accessible for irrigants, which leads for more remnants of smear layer/debris. The present experimental study was designed to evaluate the role of the size of apical root canal preparation on removal of smear layer after irrigation with EDTA solution in the model of extracted human teeth. Three groups of specimens were created, where the apical preparation was finished with #25/.08 (group A), #40/.06 (group B) and #50/.06 (group C). In general, complete removal of smear layer/debris from the scanning electron microscopically evaluated part of the root canals in 100% of the specimens was never seen in any of the groups. Total covering of treated dentinal walls by smear layer/debris has been detected in all groups (50%, 33.3%, and 7.10% in groups A, B and C respectively).

Analyses were made on the basis of the percentage of smear layer/debris-free dentinal wall surface. Significant differences between the groups were found when different criteria were used. Estimating slight+medium (0-50%) surface covering of the apical root canal walls with smear layer/debris, significantly better scores ($p=0.000$) of group C in comparison with the other groups (81%, 45.2%, and 35.7% groups C, B and A respectively) was noticed. By using the same criterion significantly better scores at a distance of 2 mm ($p=0.041$), and 4 mm ($p=0.002$) from the apical end was found in group C ($p=0.002$), while no significant difference was found at the level of 6 mm from the apical end between the three groups. However,

estimating only slight (0-15%) surface covering of the apical root canal walls with smear layer/debris, the group C showed significantly better scores at levels 4 mm (p= 0.004) and 6 mm (p=0.023), while no difference was found between groups at the apical 2 mm level. The results clearly indicate that ability of irrigation with EDTA to remove smear layer/debris from the treated dentinal walls at the apical 6 mm of the root canals and to facilitate their chemomechanical debridement, depends on the size of apical preparation.

A number of previous in vitro studies in the same model but using different parameters in root canal preparation and irrigation, have shown inconsistent findings. NaOCl, which is the most used irrigating solution for root canal therapy has been used in these investigations in concentrations ranging from 1% to 5.25%. The application time of NaOCl was 40 minutes. On the other hand, a 17% EDTA concentration, has been used for the elimination of the smear layer [1]. In most of the previous studies sectioning was done in buccolingual direction using diamond bur [8, 21]. SEM evaluation was done in most of the in vitro studies with a single picture taken showing one magnification only. In the present study two pictures were taken for the same area at two different magnifications (x500 and x1000), for better visualization of the scanned area and scoring of the samples.

A systematic review study conducted [29], confirmed that more evidence-based research is required in the area of apical enlargement efficacy. Recent researches on this topic are divided into three groups

- A number of studies suggested that apical enlargement significantly reduce microbial flora [16, 30, 33, 34], but in teeth where calcium hydroxide has been used as intracanal medicament for 1 week.
- In a second group authors suggested that enlarging the apical area above 60 MAFS had better results in microbial reduction [19], while
- Other studies indicated that the apical enlargement had no significant effect on microbial flora [35, 36, 37].

We must keep into consideration that direct comparison cannot be made due to different methodologies and varying parameter factors used in each research.

It has been well recognized that success of root canal treatment depends on number of parameters. Apical size preparation is just one factor in this multifactorial treatment outcome. Antimicrobial properties of solutions used in irrigations [33] delivery systems of irrigants [38], canal anatomy and configurations as isthmuses and curvatures [19], intracanal medicaments [16], apical patency methodology [39] and canal taper [40], have been considered among the many other parameters of chemomechanical preparation influencing the root canal treatment outcome.

In 1994 Yared et al. [35], conducted a study to evaluate the influence of apical enlargement on bacterial infection of root canals. Sixty single rooted teeth were used. The canals were flared using size 60 reamers and Gates Glidden #1, 2, 3, and 4, respectively. Half of the samples were prepared to a size 25 file and the other half to a size 40 file. Sodium hypochlorite solution 1% was used as canal irrigant. This study concluded there was no statistically significant difference noted between size 25 and 40 file groups after instrumentation in bacterial infection. Usman et al. [41], found a significant difference in smear layer removal between size 20 and size 40 GT instruments, and suggested that it was the master apical file size which enhanced better removal of smear layer rather than the cycles of irrigation. Huang et al. [42], comparing the preparation with different apical sizes 20 and 40 and tapers 0.04, and 0.08, followed by static or dynamic irrigation found that increased apical size and taper allowed better irrigation access in the root canal system.

Khademi et al. [21], reported that apical instrumentation to a 30-size file with 0.06 coronal taper is effective for the removal of debris and smear layer from the apical part of root canals. Mickel et al. [43], in a comparative study of extent of intracanal bacterial (Enterococcus faecalis) load in 100 teeth prepared by different apical size preparations with crown down technique demonstrated that a significant increase in the number of samples with negative

cultures were seen in groups of 1 to 3 files after the one file reaching to the full working length. Fornari et al. [22], conducted a study to evaluate the effect of apical enlargement on cleaning of the apical third of curved canals. Forty-four mesiobuccal canals of maxillary molars teeth were included in the study. They were instrumented to different apical sizes 30, 35, 40 and 45 with 0.02 taper, using crown-down technique in canal preparation. Samples were examined histologically, and results showed that there was a significant correlation between the amount of remaining debris and the perimeter of uninstrumented root canal dentine. The study concluded that no apical enlargement size allowed the root canal walls to be prepared completely. Apical third cleanliness could be predicted by instrument diameter. The results of the present study clearly confirmed those reported by the study of Fornari et al. [22].

Furthermore, in the recent literature there are other methodologies to study the effect of apical preparation size on irrigant flow inside a root canal. Boutsioukis et al. [44], conducted a study to evaluate the irrigant flow characteristics during final irrigation with a syringe and two different needles types, using a Computational Fluid Dynamics model. Canals were prepared up to size 25, 35, 45 and 55, all with a 0.06 taper. Side vented and flat 30G needles were used. Authors concluded that apical preparation size affected all flow characteristics: irrigant replacement in the root canal, the shear stress on the canal wall and the pressure at the apical foramen. Root canal enlargement to sizes larger than 25 appeared to improve the performance of syringe irrigation. In a previous study has been reported that the flow apical to a beveled needle placed 3 mm short of working length reached to the full working length in root canals prepared to size 35, 45 and 55 but not at 25 [45].

Saini et al. [26], conducted a study on the effect of apical preparation size in relation to the first apical binding file on the outcome of primary endodontic treatment. One hundred sixty-seven patients were included and divided randomly into 5 groups prepared to 2, 3, 4, 5, and 6 sizes larger than the FABF, respectively. The study concluded that enlargement of the canal to 3 sizes larger than the FABF is adequate, and further enlargement does not provide any additional

benefit during endodontic treatment. In this study the group of teeth with preparation of #50 size showed much better performance than the #25 and #40 size files. Our results of group B and C confirmed the findings of a recent study [8], where the apical enlargement more than size 35 and 0.04 taper with conventional irrigation allowed enhanced smear layer removal in the apical third.

Although many researches have been demonstrated the role of apical enlargement in treatment performance in both in vitro and clinical conditions, MAF determination in the root canal treatment remains a controversial topic. Classical studies have been suggested that a higher healing rate was achieved when MAFS with traditional taper of 0.2 were kept as small as possible [46, 47]. The risk of procedural errors as transportation and perforations with aggressive apical instrumentation has been reported, while the observation that the enlargement of apical root canal system did not ensure complete removal of the inner layer of dentin from all apical root canal walls or all infected necrotic pulp tissue [15] has been also confirmed in the present study. A recent study conducted by Yuan et al. [48], showed that under vertical and lateral occlusal loads, greater apical enlargement resulted in increased stress on the remaining dentinal walls, especially at the apex. Authors suggested that while increased instrumentation may allow more efficient cleaning, the use of increasingly larger files sizes should be avoided in the appropriate using of irrigants.

A number of limitations should be addressed in in vitro studies of the role of chemomechanical preparation in the cleaning and shaping of the root canals. The most important limitation is that it encounters only the straight canals, which does not reflect the many anatomical variations of the canals that the clinician may consider. Efficacy of cleaning evaluated by the number of opened dentinal tubules, but pathogens may exist in areas deeper within the dentinal wall. A factor that may also affects the present results and should be considered as limitation is the only one technique of rotary instrumentation which has been used.

It seems reasonable to suggest from the available results on the role of apical enlargement in rendering 100% bacteria free root canals and completely clean dentinal walls from smear layer/debris, remains as an extremely challenging issue in endodontic research. Further studies with advanced techniques of chemomechanical preparation, and improved methodologies of evaluation are required.

7. CONCLUSIONS

1. Complete removal of smear layer/debris from the root canals in all specimens were never found in any of the size of apical enlargement or at distance from the root apex. Total covering of treated dentinal walls by smear layer/debris has been detected in all apical enlargement groups.
2. Significant differences between the groups of teeth with different size of apical enlargement was found when various measurement criteria were performed. More particularly, significant differences between groups A and C (null hypothesis 2 was rejected) and groups B and C (null hypothesis 3 was rejected) were observed. No significant differences between groups A and B (null hypothesis 1 was confirmed) were found.
3. When slight+medium (0-50%) surface covering of the apical root canal walls with smear layer/debris was evaluated, significantly better scores of group C (MAF #50) in comparison with the other groups was seen. Significantly better scores at a distance of 2 mm and 4 mm but not at 6 mm from the apical end were found in group C.
4. When only slight (0-15%) surface covering of the apical root canal walls with smear layer/debris was evaluated, the group C showed significantly better scores, in comparison with the other groups, at levels 4 mm and 6 mm but not at a distance of 2 mm from the root apex.
5. The present results clearly indicate that the ability of irrigation to remove smear layer/debris from the instrumented straight root canal at the apical part of the canals is enhanced with increasing the size of apical preparation with the Protaper rotary system.

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