The Effect of Apical Size Enlargement and Irrigant Activation on Elimination of Radicular Pulp Tissue: A Histological Study

Thesis Submitted in partial fulfillment of the requirements for master's degree in endodontics.

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Dedication

This work is lovingly dedicated to…….

*My **parents**, your unconditional love and gracious care made all the difference in my life.*

*My beloved **wife and sons**, you are the angels of my life and the best support.*

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First and foremost, I begin by praising **Allah** the all-knowing, the all merciful, and I bear witness that Allah, my Lord, alone is worthy of being worshipped. For without my Lord's grace, guidance and support none of this would have been possible.

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1. Introduction

The ultimate goal for root canal treatment of teeth is to remove bacteria and microorganisms from the root canal and to obtain a debrided and disinfected canal space and make it able to be filled efficiently[1]. In order to achieve this purpose, endodontic files are used to prepare the root canal, remove pulp tissue and debris, and ensures easy delivery of the irrigants and medicaments to the apical third which usually has a complex anatomy[2]. Because of this complexity, it was found that depending on endodontic instruments only to completely debride the root canal is insufficient[3,4]. Here comes the need to introduce chemical irrigants inside the root canal to dissolve pulp tissues and remnants, clean inaccessible areas untouched by the instruments and prevent debris resulting from the friction between endodontic files and root canal walls to accumulate at the apex causing re-infection[5]. Sodium hypochlorite (NaOCl) has been considered the gold standard amongst different irrigation solutions due to its potent antimicrobial action, as well as its ability to dissolve organic substances and inactivate bacterial endotoxins[6].

Root canal debridement using conventional syringe irrigation (CSI) has a limited efficacy in canal disinfection especially in the apical region as the flow of the irrigants is limited and unable to penetrate canal irregularities such as lateral canals and apical deltas[7]. In order to enhance the action of the irrigation solutions in canal debridement, many activation techniques were introduced to enhance the efficacy of the irrigating solutions such as manual dynamic activation (MDA), sonic activation, laser activated irrigation (LAI), apical negative pressure activation and passive ultrasonic irrigation (PUI). PUI is

considered one of the most popular techniques amongst the different activation techniques[8,9].

PUI is a non-cutting irrigation protocol that relies on the transmission of acoustic energy from a smooth wire or an oscillating file that stirs the irrigating solution in the main root canal driving it to remote untouched areas[10]. This irrigation technique induces two physical phenomena: acoustic streaming and cavitation of the irrigating solution which creates bubbles in the liquid and when these bubbles collapse near the canal walls, they create strong shockwaves and powerful irrigant jets increasing the applied shear stress and improving the cleaning efficiency of the irrigant[11]. Moreover, part of the kinetic energy of the ultrasonic file is transformed into heat leading to a temperature rise of the irrigant in the canal that furtherly enhances its antimicrobial role[12].

Very little research has been done to evaluate to which extent the canal should be enlarged without unneeded removal of dentinal structures and weakening the root or this goal can be achieved with a smaller apical preparation size with the aid of irrigant activation technique.

2. Review of literature

Section outline:

2.1. The paradigm of apical size enlargement:

2.1.1 The relation between apical enlargement and root canal debridement.

2.1.2 The relation between apical enlargement and irrigation efficiency.

2.1.3 The relation between apical enlargement and radicular structure.

2.1.4 The relation between apical enlargement and healing outcomes.

2.2. Effect of irrigant activation on pulp tissue and hard tissue removal:

2.2.1 Different activation methods.

2.2.2 Passive ultrasonic irrigation.

2.3. Methods of evaluation of pulp tissue remnants.

2. Review of literature

2.1. The paradigm of apical size enlargement:

Apical size enlargement has always been a point of controversy that has not been resolved yet. Many authors believe that increasing the apical preparation size would favorably affect the root canal cleanliness and the healing outcomes whilst other think that the role of apical enlargement is minimal and its drawbacks overweigh its benefits.

2.1.1 The relation between apical enlargement and root canal debridement:

Rollison et al.[13] in 2002 assessed the effect of apical enlargement on bacterial removal from the root canal in mandibular molars. Samples were inoculated in a specific amount of ^{3}H-thymidine–labeled Enterococcus faecalis (E. faecalis) for 5 days. Group 1 samples were instrumented up to size #35/.04, and those of group 2 were prepared up to size #50/.02. After instrumentation, paper points were inserted in each canal and radioactivity of the collected medium was measured using liquid scintillation spectrometry. The result was that ^{3}H-thymidine recovered from group 2 was significantly higher than group 1. They suggested that apical enlargement up to file size #50/.02 was more effective in bacterial removal than using file size #35/.04.

Tan et al.[14] in 2002 compared the quality of root canal debridement after apical enlargement using conventional stainless steel K-files and rotary LightSpeed instruments through histological evaluation. Teeth were divided as follows: group 1: master apical file (MAF) was 3 sizes larger than the first apical binding K-file (FABF) without coronal flaring, group 2: MAF was 3 sizes larger than FABF after coronal flaring, group 3: root canals prepared with LightSpeed

rotary instruments and MAF was determined as the smallest file that required a minimum of 12 pecks to reach the working length. The largest apical preparation size was achieved with LightSpeed group. Results revealed that LightSpeed instrumentation resulted in significantly cleaner canal walls that were planed without any remnants of pulp tissue or dentin debris remaining compared with instrumentation using K-files.

Albrecht et al.[15] in 2004 evaluated the effect of instrumentation size and taper of the root canal on debris removal through histological examination. Anterior teeth and bicuspids were collected and divided in bilaterally matched pairs. Every pair was sequentially prepared using files with taper .02, .04, .06, .08 and .10 with one tooth of the pair prepared up to file #20 and the other one prepared up to file #40. It was found that, in all teeth instrumented up to #20 regardless of the taper, there was a significant higher amount of debris than those prepared to #40. The only exception was teeth tapered to .10 as there was no significant difference between file #20 and #40.

Khademi et al.[16] in 2006 determined the minimal optimal preparation size needed for an effective removal of debris and smear layer from the apical third of the mesiobuccal root canals of lower first molars. The teeth were divided into 4 groups according to the final largest instrument used (#20, #25, #30, #35) and then scanned under a scanning electron microscope (SEM). They found that using file #30/.06 taper is sufficient for effective cleaning of the apical third of the root canal.

Mickel et al.[17] in 2007 assessed the relation between apical size and extent of intracanal bacterial load. Teeth were contaminated with an inoculum of Enterococcus faecalis then they were prepared using

crown down technique and the first file to reach the full working length was recorded as the crown-down file (CDF). Teeth were then divided into 3 groups according to the MAF size: group 1: MAF was one file size larger than CDF (CDF+1), group 2: CDF+2, and group 3: CDF+3. In the control group, negative controls were not inoculated with bacteria, whereas positive controls were inoculated after cleaning and shaping the canals. Bacterial extract was collected from each sample and counted. The results have shown that bacterial counts decreased between the CDF+1 to CDF+3 groups. Negative controls yielded no bacterial colonies, whereas positive controls yielded significantly higher bacterial counts.

Marinho et al.[18] in 2012 investigated the influence of various apical file sizes on the endotoxin level in the root canals. Escherichia coli endotoxin was introduced in lower bicuspids. After the incubation period, endotoxin samples were collected using absorbent paper point from root canals before instrumentation and after introducing each file size of #25/.06, #30/.05, #35/.04 and # 40/.04. Quantitative analysis of endotoxin levels revealed that endotoxin levels were significantly reduced after using file #35/.04, and #40/.04. It was concluded that larger apical size preparation has led to improved endotoxin content removal.

Akhlaghi et al.[19] in 2013 determined the impact of MAF size on the debridement of the apical third of curved canals of lower first molars using SEM. Teeth were divided into 6 groups and prepared to file sizes #25/.04, #25/.06, #30/.04, #30/.06, #35/.04 and #35/.06. The results demonstrated that samples prepared to file size #35/.06 showed 100% canal debridement. The percent was non-significantly less in groups with file sizes #35/.04 and #30/.06. No adequate cleaning was

obtained in groups with file #25/.06 and #25/.04. They concluded that reaching file size #30/.06 is the minimal suitable size for optimal cleaning of the root canal.

Xu et al.[20] in 2018 compared the removal efficacy of dentinal debris in mandibular first molars prepared to various apical sizes. Teeth were prepared to an apical size of #25/0.04, #30/0.04, #35/0.04, and #40/0.04. Pre and post-operative micro computed tomographic (micro-CT) scanning was performed to all samples and both scans were superimposed to identify and quantify the remaining hard-tissue debris. Results showed that the amount of accumulated hard-tissue debris was significantly less in with MAF #40/.04 than in the other groups. There were no significant differences between Groups #30/.04 and #35/.04.

Laslami et al.[21] in 2018 investigated the relationship between the apical preparation diameter and the apical sealing ability. Teeth were divided according to the apical preparation size: group 1: samples were prepared up to size #20, group 2: prepared up to size #30, Group 3: prepared up to size #50. All samples were obturated then immersed in 1% methylene blue for 48 hours. After removal from the dye solution, roots were longitudinally split and photographed to determine the depth of dye penetration and microleakage. The results revealed there are no significant differences in the apical leakage between the three different apical preparation sizes (#20, #30 and #50).

Plotino et al.[22] in 2019 evaluated the effect of apical enlargement on root canal cleanliness. Mandibular molars were divided according to the final file size used during instrumentation: size #20/.04, #20/.06, #25/.04 or #25/.06. Roots were longitudinally split and smear layer and debris were investigated under SEM. It was found that smear layer removal in the apical third was significantly more efficient with

file size #25/.04 and #25/.06. They concluded that file size #25 results in a cleaner root canal than file size #20.

Stringheta at al.[23] in 2021 evaluated the amount of pulp tissue remnants and the area of untouched canal walls after apical preparation to different sizes using histological assessment. Mandibular molars were divided according to the final file size used either #30 or #40 using different file systems and different irrigation protocols. The results revealed that apical enlargement up to #40 resulted in lower amount of pulp remnants and unprepared canal walls than #30 regardless of the file system and the irrigation technique used.

Almadi et al.[24] in 2023 evaluated the effect of apical enlargement on smear layer removal while using photon-induced photoacoustic streaming (PIPS) agitation technique. Teeth were divided into 3 experimental groups and one control group according the final apical preparation size: group 1 samples prepared up to F1 +PIPS protaper files, group 2: up to F2 + PIPS, group 3: up to F3+PIPS and group 4: F3 + CSI. Samples were then scanned under SEM and the amount of smear layer remaining was measured. The results revealed that preparing samples to file F3 removed greater amount of smear layer, and those prepared to file F1 had the largest amount of smear layer in their root canals.

2.1.2 The relation between apical enlargement and irrigation efficiency:

Boutsioukis et al.[25] in 2010 evaluated the influence of apical diameter enlargement on the flow pattern of irrigating solution in the canal using computational fluid dynamics (CFD) model. Geometrical frusta were used to simulate the root canal with apical diameters of 0.25,

0.35, 0.45 and 0.55 and taper of 6% in all of them. Side-vented needles and flat needles were positioned 3mm short of the full length. CFD model was used to evaluate irrigant replacement rate, shear stress and apical pressure. They found that increasing the apical size above #25 has significantly increased the irrigant replacement and reduced the apical pressure and shear stress especially with the flat needle and ensured a sufficient space between the needle and canal wall to allow reverse flow of the irrigant to the canal orifice.

Brunson et al.[26] in 2010 determined the influence of the size and taper of the MAF on the irrigant ability to reach the full length of the root canal. Single-rooted teeth were divided into two groups. In group 1, teeth were prepared with the file sequence #30/.06, #35/.06, #40/.06 and #45/.06. In group 2, teeth were prepared with file sequence #40/.02, #40/.04, #40/.06 and #40/.08 and teeth of both groups were irrigated after each file use using micro cannula and the amount of NaOCl suctioned under negative pressure was collected and measured. They found that enlarging the file size from #35 to #40 has led to 44% increase in the irrigant volume while increasing the file size to #45 resulted in only 4% increase in irrigant volume. Increase in taper from #40 / .02 to #40 / .04 had the greatest effect on the irrigant volume. They concluded that file size #40 /.04 is optimal for adequate delivery of irrigants and preservation of tooth structure.

Merino et al.[27] in 2012 evaluated the penetration ability of irrigants up to working length after root canal preparation with 0.04 and 0.08 taper instruments. The samples were divided into group 1, canals instrumented to size #30/0.04, and group 2, instrumented to size #30/0.08. Both groups were irrigated with a contrast solution and divided in 2 subgroups groups: 1A and 2A were activated with PUI,

whilst groups 1B and 2B were activated with sonic irrigation. Radiographs were taken to evaluate the penetration of the contrast solution up to working length. Results revealed that with 0.04 taper, PUI resulted in significantly greater irrigant penetration when compared with sonic activation Increasing taper to 0.08 resulted in no significant differences between the both activation systems.

Srikanth et al.[28] in 2013 determined the effect of apical enlargement on the penetration depth of the irrigant to the apical third. Maxillary first molars were collected and divided into 4 tested groups according the MAF size: #20, #25, #30 and #35/.06 as well as a negative control and a positive control group which were prepared to MAF #40/.06. After instrumentation procedures, the 4 tested groups and the positive control group received a final irrigation of 5ml of smear clear and 5ml of NaOCl, but the negative control group was irrigated with 5ml of NaOCl only. SEM investigations have shown that negative control group samples were covered with smear layer while the positive control group samples were devoid of smear layer and debris. It was also found that groups 1 and 2 had much higher amount of debris than groups 3 and 4. It was concluded that enlarging to, at least, file size #30 is mandatory to ensure a proper penetration of the irrigating solution to the apical third.

de Gregorio et al.[29] in 2013 compared the effect of increasing the size and taper of MAF on the irrigant volume reaching the full length of root canals with different curvatures. Teeth were prepared to different MAF sizes and tapers: #35/.06, 40/.04, #40/.06, #45/.04, #45/.06. They followed the same protocol of measuring irrigant volume as what **Brunson et al.**[26] did. The results revealed that there was a significant increase in irrigant volume when MAF size increased from

#35/.06 to #40/.04. Also, the increase in taper from 40/.04 to #40/.06 resulted in a significant increase in the irrigant volume. They concluded that, regardless of the root curvature, MAF #40/.06 would adequately allow the irrigants to reach the full working length.

Butcher et al.[30] in 2019 determined the effect of apical enlargement on the efficiency of the CSI to remove smear layer in the apical third. Lower premolars were divided into 5 experimental groups according to MAF size: #25, #30, #35, #40 or #45 and 1 control group which was prepared to file size #45. Experimental groups were irrigated with 2.5% NaOCl while the control group was irrigated with distilled water only. Upon investigation under SEM, it was found that smear layer removal in the apical third was noticeably improved after preparing to file size larger than #35 whereas there was no significant difference in the coronal and middle thirds in all groups.

Sujith et al.[31] in 2021 evaluated the irrigant flow and apical pressure in simulated canals with different tapers and apical preparation sizes. Teeth were divided according to the final file used: group 1: up to file #30/.06, group 2: up to file #30/.04, group 3: up to file #25/.06 and group 4: up to file #25/.04. Teeth were scanned using Cone Beam Computed Tomographic (CBCT) scanning and computer-aided design models were obtained and computational fluid dynamics technique was performed to assess the irrigant flow and apical pressure of the irrigating solution. The results revealed that file #30/.06 was accompanied with the most efficient flow of the irrigant and the lowest value of apical pressure.

2.1.3 The relation between apical enlargement and radicular structure:

Weiger et al.[32] in 2006 determined the minimal rotary file size diameter which allows complete cutting of the inner layer of root dentin at the level of 1mm and 2mm far from apex in upper and lower first molars. The results demonstrated that in distal or palatal root canals an apical enlargement of 0.4mm larger than the FABF size is needed but only 0.3mm in smaller canals (mesiobuccal, distobuccal and mesiolingual canals). They concluded that in wider canals, apical preparation size is recommended to be at least 8 file sizes larger than FABF and 6 file sizes larger than FABF in smaller canals.

Hecker et al.[33] in 2010 investigated the recommended apical preparation size of premolar root canals using non-tapered, non-cutting instruments. They found that enlarging the apical preparation size 0.3mm larger than the diameter of the largest special instrument reaching the full length resulted in 75% complete preparation of canal walls in upper premolars with 2 canals and lower premolars. In upper premolars with 1 canal, they found that enlarging the apical preparation size 0.4mm larger than the largest special instrument reaching the full length is needed to process 63% of the canal walls. They concluded that the optimal MAF size should be 6 sizes larger than the FABF in upper premolars with 2 canals and lower premolars, while in upper premolars with a single canal the apical preparation size needs to be at least 8 times larger than the FABF.

Elayouti et al.[34] in 2011 evaluated the relation between enlarging the MAF size and radicular dentin removal in curved root canals. Mesial roots of molars with curvatures between 25°-50° were scanned using micro-CT scanning then they were prepared to MAF #50

using 3 different file systems: MTwo file system, nickel-titanium hand files and ProTaper files. A micro-CT scanning was obtained after file #25, 30, 40 and 50. By measuring the percentage of prepared areas and amount of radicular dentin removed, they found that increasing the MAF from #30 to #50 did not significantly increase the prepared area with all file systems, but it has unnecessarily led to excessive removal of dentin especially with ProTaper files.

Moradi et al.[35] in 2014 investigated the relation between master apical file size and canal transportation incidence in severely curved canals. Mesial roots of lower first molars with curvatures ranging from 45º-60º were collected and scanned using CBCT scanning prior to instrumentation then they were divided to 3 groups according to MAF size: Group A: MAF size #20, group B: MAF size #25 and group C: MAF size #30. A post-operative CBCT scanning was performed to assess the apical transportation and it was found that there was no significant degree of apical transportation in different curvatures when enlarging the preparation up to file #30.

Pérez et al.[36] in 2018 evaluated how apical enlargement during root canal preparation could affect the remaining dentin thickness and surface area of unprepared canal walls using micro-CT. 30 mandibular incisors were prepared sequentially with 4 rotary Hyflex files larger than FABF of each tooth and they were scanned after each file use. They found that there was a significant reduction in unprepared areas as well as dentin thickness after each file use, however dentin thickness was always larger than 1mm. They concluded that enlarging the apical portion of the root canal 4 sizes larger than FABF has a favorable effect on removal of unprepared areas.

Doğanay et al.[37] in 2021 investigated the effect of MAF size and taper on fracture resistance of lower incisors. Samples were divided into a negative control group and 6 experimental groups according to MAF size: 25/.04, 25/.06, 25/.08, 30/.04, 30/.06 and 30/0.08. By measuring the fracture resistance of each sample, it was non-significantly reduced by increasing the taper of the final file used from 0.04 to 0.06, while it was significantly reduced when the taper of MAF change from 0.04 to 0.08 without changing the size. Also, there was a significant decrease when MAF changed from #25/.04 to #30/.06 or from 25/.06 to #30/.08. They claimed that larger tapers of MAF could lead to higher fracture risk.

2.1.4 The relation between apical enlargement and healing outcomes:

Saini et al.[38] in 2012 determined the impact of apical size enlargement on the root canal treatment outcome. 167 patients were included in the study and divided into 5 groups in which canals were prepared to 2, 3, 4, 5 and 6 file sizes larger than the FABF. After 12 months, the treatment outcome was evaluated radiographically and clinically. They found that there was a significant increase in the number of healed cases when 3 file sizes larger than FABF were used and that the effect of further apical size enlargement had a minor effect on the healing progress.

Silvestrin et al.[39] in 2016 determined the correlation between the apical size enlargement and the bacterial leakage of the obturated root canals. They divided 125 teeth into 5 groups according to the largest apical file size used (#30, #40, #50, #60 and #70) then obturated the canals with gutta-percha and sealers using the warm vertical compaction technique. The results showed that, after 112 days, the time

needed for bacterial leakage to occur was inversely proportioning with the apical preparation size with the fastest leakage in samples with apical file size #70 and slowest leakage in the group of #30. They concluded that a significant increase in the apical leakage occurs when the apical size is larger than #60.

Jara et al. [40] in 2018 evaluated the apical periodontitis healing in rats after apical enlargement using several file sizes. Pulp exposure was initiated in right and left first molars in 24 rats and left for 3 weeks to induce apical periodontitis. Root canal treatment was performed in one side and rats were divided into 3 groups according to the apical enlargement size: MAF #20, #25 or #30. The other side remained untreated and acted as the control group for each rat. Radiographic and histologic examination showed better healing in samples prepared to file size #30. They concluded that preparing root canal to MAF size #30 resulted in a more favorable healing outcome.

Fatima et al.[41] in 2021 investigated the influence of MAF size and taper on post-operative pain and healing process after endodontic retreatment of asymptomatic failed primary root canal treatment in lower first molars. Patients were divided into 2 main groups and 2 subgroups. In group 1: root canals were enlarged 2 sizes larger than FABF with 4% taper in subgroup1A, while in subgroup 1B, file taper was 6%. Group 2 was prepared 3 sizes larger than FABF and the same subgrouping was done depending on the taper. Post-operative pain assessment has shown no significant difference between patients. Radiographic assessment has shown a significantly lower success rate in subgroup 1A. It was concluded that enlarging the MAF 2 sizes larger than FABF with 4% taper is insufficient for achieving a successful healing outcome.

2.2. Effect of irrigant activation on pulp tissue and hard tissue removal:

2.2.1 Different activation methods:

Thapak et al.[42] in 2021 compared the efficiency of different irrigant activation techniques in smear layer removal using SEM. Mandibular premolars were prepared up to file F3 protaper rotary file and assigned to three experimental groups according to the activation technique used: MDA, sonic irrigation (EndoActivator), and Er:YAG laser. It was found that the Er:YAG laser group showed significantly lower amount of smear layer in the apical third as compared to all other groups. Sonic activation protocol resulted in better cleaning efficacy at the apical area compared to MDA.

Karunakar et al.[43] in 2021 determined the efficacy of smear layer removal after using different irrigant agitation techniques. Teeth were prepared up to file F3 protaper rotary file then they were divided into 4 groups according the final irrigation technique used: EndoVac, PUI, diode laser, or conventional irrigation. samples were scanned under SEM to evaluate the amount of remaining smear layer. The results showed that diode laser and EndoVac were more efficient in smear layer removal compared with PUI.

Aydin et al.[44] in 2023 determined the pulp tissue debridement efficacy among different activation methods using histological assessment. Teeth were mechanically prepared using different file systems and then divided according to the final irrigation protocol: Endoactivator (sonic activation), EndoUltra (ultrasonic activation) or CSI were used. The results revealed that CSI left greater amount of debris than both activation techniques and the EndoUltra was the most effective technique in root canal debridement.

2.2.2. Passive ultrasonic irrigation:

Lee et al.[45] in 2004 compared the debridement efficiency of smear layer removal between CSI and PUI. The teeth were instrumented, longitudinally split into two halves, and an artificial groove was created in the inner canal wall to mimic unprepared canal extensions and dentin debris were introduced in those artificial grooves then the two halves were re-assembled to activate the irrigant using either CSI or PUI protocols. The two halves were re-separated and imaged using a digital microscope at 40X magnification and a digital camera then the amount of remaining debris was measured. Results revealed that that PUI has led to a significantly lower amount of remaining debris than CSI.

While **Gutarts et al.**[46] in 2005 evaluated the effectiveness of PUI and CSI on radicular pulp tissue elimination using histological evaluation. Endodontic treatment was carried out in patients with vital teeth planned for extraction for other reasons and the irrigant was done using PUI or CSI protocols. Teeth were then extracted and processed to calculate the area of remaining pulp tissue remnants compared to the whole area of the canal. The isthmuses between canals were traced separately from the primary root canals and their total area and the area of their remaining pulpal tissue were recorded. Results revealed that PUI resulted in cleaner canal space at the apical level and isthmus than CSI. There was no significant difference in canal cleanliness using the two techniques at 3mm level from the apex.

Burleson et al.[47] in 2007 evaluated the effect of PUI in root canal debridement in patients with necrotic mandibular molars using histological evaluation. Endodontic treatment was performed with the same instrumentation technique in all patients but the final irrigation

was done in one group using CSI while in the other group, an ultrasonic needle was used to activate the irrigant for 1 minute. Immediate extraction was then carried out and teeth were processed for histological examination to evaluate the amount of remaining debris and bacteria in the main canals and isthmuses. It was found that there was a significant improvement in canal and isthmus debridement when using ultrasonic needle irrigant activation.

De Groot et al.[48] in 2009 compared between the effectiveness of CSI, PUI and LAI in debris removal in maxillary canines. After teeth instrumentation and splitting, artificial grooves were created in the inner canal wall, wet dentin debris were placed inside the grooves and teeth were re-assembled. Final irrigation was done using the above-mentioned techniques then teeth were separated and imaged with a digital camera under 40X microscope to evaluate remaining debris. Results have shown that LAI was significantly more effective in debris removal than PUI or CSI. It was also found that PUI was significantly better than CSI in canal debridement.

De Moor et al.[49] in 2009 evaluated the cleaning efficiency of PUI, LAI and CSI in dentin debris removal from the root canal. The same methodology of teeth instrumentation, splitting, grooving, dentin debris placement and different irrigation protocol using PUI, LAI or CSI were performed according to **Lee and De Groot**[45,48]. Teeth were re-separated and images of the artificial grooves were captured using a digital camera under x40 magnification to compare the amount of dentin debris before and after final irrigation. The results came in line with **Lee and De Groot** work[45,48]. They concluded that LAI is the most effective irrigant activation method followed by PUI in smear layer

removal and both techniques resulted in significantly cleaner canals than CSI.

Van der Sluis et al.[50] in 2009 investigated the efficiency of PUI on canal debridement comparing continuous and intermittent flushing methods. Root canals were split, artificial grooves were created, dentin debris were produced and grooves were captured under 40X magnification. After mechanical instrumentation, teeth were divided into 5 groups: group 1 and 2: root canals were irrigated using ultrasonic needle with continuous supply of irrigant for 1.5 minute and 3 minutes respectively. Group 3 and 4: intermittent irrigation with plastic syringe then ultrasonic activation for 1 and 3 minutes respectively. Group 5: irrigation was done using CSI. Teeth were re-split and captured to evaluate remaining debris. Results have shown that intermittent flushing for 1 minute was as efficient as continuous and intermittent irrigation for 3 minutes. Continuous flow for 1.5 minutes was less effective and all of them were better than CSI.

Rödig et al.[51] in 2010 evaluated debris removal efficiency of PUI compared with Vibringe sonic activation device and CSI. Teeth were split and 2 artificial grooves were created in the coronal and apical thirds and dentin debris were placed in the grooves and grooves were then photographed under 30X magnification. After teeth reassembling and instrumentation, final irrigation was done using PUI, Vibringe or CSI. Teeth were separated and grooves were re-imaged to assess the remaining debris. It was found that PUI was significantly better in debris removal than Vibringe system and CSI.

Rödig et al.[52] in 2010 also compared the PUI efficiency in hard tissue debridement with RinsEndo device which depends on the hydrodynamic pressure of irrigants. Premolars were divided into 3

groups according to the final apical file size used: #30/.02, #40/.02 or #50/.02. Teeth were split and artificial grooves and cavities were done in the canal wall to simulate root canal irregularities then dentin debris were placed inside them. Different irrigation protocols were sequentially done for the 3 groups: CSI, RinsEndo then PUI. After each protocol teeth were re-split, captured under 30X magnification to evaluate the amount of remaining debris then grooves were filled again with dentin debris. It was found that PUI was significantly better than RinsEndo in groups #40/.02 and #50/.02 but the difference was statistically non-significant with #30/.02. RinsEndo removed debris significantly better than CSI.

Al-Ali et al.[53] in 2012 investigated the difference between CSI using NaOCl and 3% H_2O_2, CanalBrush agitation and PUI on soft tissue and smear layer removal from the root canal. Part of the samples of each group were histologically examined for pulp tissue removal assessment while the rest were scanned under SEM to evaluate smear layer removal. The results revealed that soft tissue removal was more efficient when using 3% H_2O_2 than PUI. SEM investigations showed superior ability of PUI and CanalBrush in smear layer removal compared with CSI.

Yoo et al.[54] in 2013 assessed cleaning efficiency of PUI in comparison with different irrigation techniques in canal and isthmus mandibular molars. Mesial roots of mandibular molars were instrumented and then divided according to the final irrigation technique used: Group 1: CSI, Group 2: PUI with #15 ultrasonic k-file, group3: continuous ultrasonic irrigation (CUI) and group 4: Endovac system irrigation. Upon histological examination at 1, 3 and 5mm from the apex, it was found that Endovac and CUI groups resulted in a

significantly better canal debridement than PUI and CSI in the level of 1 mm, as well as significantly cleaner isthmuses than PUI at the level of 3 mm and CSI at all levels. They concluded that Endovac and CUI have a better cleaning efficacy than PUI in complex root canal systems.

Liang et al.[55] in 2013 compared the healing outcome of periapical lesions after root canal treatment between CSI and PUI. The healing outcome was assessed after a follow up period by CBCT scanning. It was found that periapical healing percentage among patients treated using PUI is non-significantly higher than those treated using CSI. It was concluded that both techniques have equally led to periapical healing.

Vinhorte et al.[56] in 2014 assessed the impact of PUI on debris removal from the root canal in lower incisors. Mechanical preparation was performed in all teeth and they were then divided according to the final irrigation protocol applied: Group 1 received 1mL. of NaOCl in the canal and remained for 1 minute. In group 2, 1mL. of NaOCl was introduced in the canal and ultrasonic agitation was done for 1 minute. Upon histological examination, it was found that PUI resulted in a significantly greater debris removal from the root canal space.

Tang et al.[57] in 2015 determined the effect of PUI on post-operative pain and treatment success rates after endodontic treatment. Pain was assessed after treatment using visual analogue scale (VAS) and clinical examination. Success rates were evaluated through clinical and radiographic examination after 6 and 12 months recalls. It was found that post-operative pain was significantly reduced in patients who received treatment using ultrasonic irrigation than those with CSI. Regarding success rates, the results were similar to those of **Liang et**

al.[55] as there was better treatment outcomes with PUI but it was statistically non-significant.

Neelakantan et al.[58] in 2016 investigated isthmus debridement ability of PUI compared with CSI, MDA and apical negative pressure with continuous warm activated irrigation system (CWAIS) in mandibular molars. After instrumentation and applying final irrigation of all groups, histological examination of the samples at 1, 3 and 5mm from the apex revealed that CWAIS has shown significantly less pulp tissue remnants than other groups at all levels. Another finding was that MDA resulted in cleaner isthmuses than PUI and CSI at 1 and 3mm from the apex.

Rödig et al.[59] in 2019 evaluated hard tissue debridement with PUI, sonic activation or CSI using micro-CT scanning. Pre-operative micro-CT scanning was done before mechanical preparation of the teeth. After instrumentation, teeth were divided into 4 groups according to the final irrigation technique: PUI, sonic activation with EDDY device, Sonic activation with Endoactivator and CSI. Post-operative micro-CT scanning was carried out and the amount of hard tissue remnants were compared with the pre-operative scan. They found that there was no significant difference in canal cleanliness between sonic and ultrasonic activation and manual irrigation.

Jesus et al.[60] in 2019 assessed the relation between different irrigation protocols and periapical healing and the production of osteopontin, tumor necrosis factor-α inflammatory mediators. Intentional apical periodontitis was induced in dogs' teeth then endodontic treatment was carried out using different irrigation techniques: negative apical pressure, PUI and CSI. After 180 days, dogs were euthanized. Radiographic evaluation of periapical repair revealed

that there was no significant difference between different groups. Immunohistochemical evaluation also showed relatively same results regardless of the irrigation protocol used.

Verma et al.[61] in 2020 evaluated healing outcomes in patients with chronic apical periodontitis when using CSI, PUI and LAI. Patients were re-evaluated through clinical examination of signs and symptoms and radiographically using CBCT at 6 and 12 months after the treatment to evaluate changes in the periapical index (PAI). They found that both of PUI and LAI were related to significant decrease in the PAI without a significant difference between them. They concluded that PUI and LAI have led to significant higher healing rates than CSI.

Wigler et al.[62] in 2023 compared the efficiency of PUI and sonic activation on smear layer removal in straight oval canals. Teeth were prepared up to file #40/.04 then they were divided according to the irrigation activation technique: group 1: EDDY sonic activation, group 2: endosonic PUI, group 3: irrisafe PUI, and group 4: CSI. The samples were scanned under SEM and the amount of smear layer was assessed. The results revealed that endosonic PUI was less effective in smear layer removal than the other activation methods, but all activation methods were more effective than CSI.

2.2.3. Methods of evaluation of pulp tissue remnants:

Fornari et al.[63] in 2020 evaluated root canal cleanliness after preparation to different apical sizes using histological evaluation. Teeth were divided according to the apical preparation size to file #40/.06 or #45/.02. They were then decalcified and 6-µm-thick transverse sections were obtained from the apical 5-mm of the root. Sections were stained with hematoxylin and eosin stain and the images were captured and imported to image analysis software for morphometric analysis. The

cross-sectional area of the root canal and remaining pulp tissue were measured to calculate the percentage of remaining pulp tissue and debris. Outlines of the canal perimeters were traced to determine the surface area touched by the instruments.

Alcota et al.[64] in 2021 evaluated the effect of irrigant activation and apical preparation on pulp tissue debridement using histological examination. After biomechanical preparation of the teeth, they were fixed, decalcified and then perpendicular cross-cut 5-µm-thick sections were obtained. Images were captured and the amount of remaining pulp remnants was evaluated with a 0 to 3 score scale. Score 0 referred to canals with organic remnants and predentin filling the whole canal lumen, score 1: organic remnants are present in most parts of the canal, score 2: relatively clean root canals with pulp remnants in the canal peripheries, and score 3 refers to an absolutely clean root canal.

Ozlek et al.[65] in 2023 evaluated the amount of remaining pulp tissue through histological assessment after using different irrigant activation techniques: PUI, LAI and intracanal heating. After biomechanical preparation of the samples, they were fixed in 10% neutral buffered formalin for 48 hours, decalcified with 5% nitric acid solution. After decalcification, samples were cut into 5-µm-thick longitudinal sections by rotary microtome and stained with hematoxylin-eosin stain. The sections were viewed under light microscope at 20X and 40X magnifications. The images were placed on image analysis software and the percentage of the remaining pulp tissue was calculated.

3. Aim of the study

The aim of this study is to determine the influence of different apical preparation sizes and irrigating solution activation on removal of pulp tissue remnants through histological assessment. The null hypothesis stated that there will be no difference between the tested groups.

4. Materials and Methods

Section outline:

4.1. Collection of teeth and fixation of the pulp tissue.

4.2. Selection and preparation of the teeth.

4.2.1. Construction of the molds and arrangement of the teeth.

4.2.2. CBCT Scanning of the teeth.

4.3. Grouping of the samples.

4.4. Cleaning and instrumentation of the root canals.

4.5. Histological evaluation.

4.6. Statistical analysis of the data.

4. Materials and methods

4.1 Collection of teeth and fixation of the pulp tissue:

Fifty vital asymptomatic mandibular premolars with fully formed root apices were collected from Faculty of Dental Medicine, Boys, Cairo, Al-Azhar University to be used in this study. The teeth were extracted as a part of an orthodontic treatment plan. The patients' ages were ranging from 18 to 40 years old to obtain similar sizes of teeth and pulp chambers. Prior to extraction of the chosen teeth, vitality testing was carried out to all teeth to ensure that all of them contain healthy pulps. Pulp vitality assessment was performed by measuring the level of oxygen saturation of the teeth using pulse oximeter CMS60C (CONTEC, Qinhuangdao, Hebei Province, China) which is consisted of monitor, central processing unit (CPU) and probe (light-emitting diode and sensor). The probe of the pulse oximeter was placed on the middle third of the crown of the tooth so that the light emitted from the diode passes from the buccal surface to reach the sensor on the lingual surface. The probe was held on the tooth surface using a special holder with two arms and a spring to hold the diode and the sensor parallel to each other on the tooth surface (fig.1).

Immediately after extraction, the soft tissues attached to the external surfaces of the roots were removed by scrapping with a curette (fig.2). Pulp tissue of the teeth was fixed by opening a conservative access cavity in each tooth using a size #3 round bur (Mani® carbide bur, 001/012, Mani inc., Togichi, Japan) mounted on a high-speed handpiece under water cooling (T3 turbine, T3 mini, Dentsply Sirona, Bensheim, Germany). Injection of 10% neutral buffered formalin (Al-Gomhoria Company for medical supplies, Cairo, Egypt) was done under pressure through the access cavity using a luer-locker plastic syringe and 31-gauge beveled

needle (BD MicroFine™, Becton-Dickinson, Wokingham, UK) until the formalin comes out from the apical foramen (fig.3). The teeth were immediately immersed in individual vials containing 10% buffered formalin for 48 hours. Teeth were then removed from the fixative solution and thoroughly rinsed with cold distilled water for 5 minutes then stored in 70% ethanol (Al-Gomhoria Company for medical supplies, Cairo, Egypt) until the time of use [66].

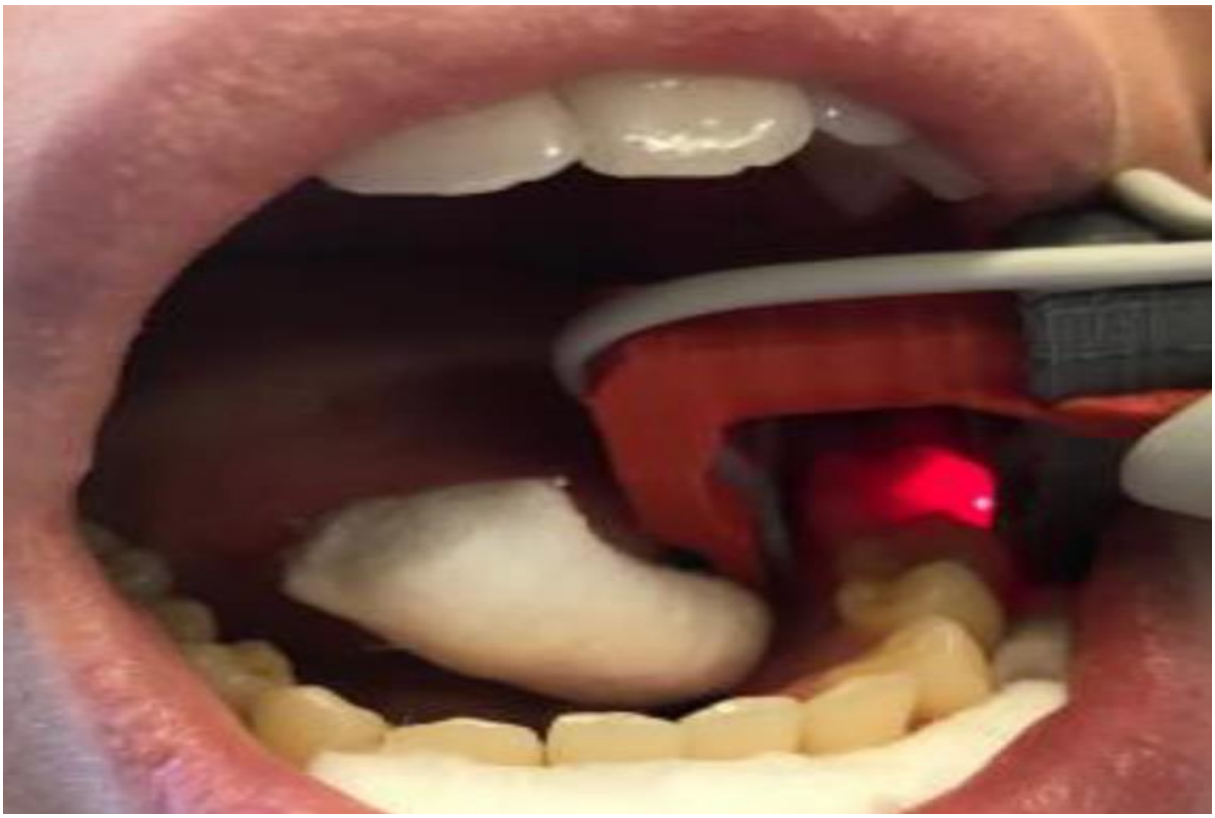

Fig. (1): a photograph showing vitality testing of the mandibular premolars using pulse oximeter device.

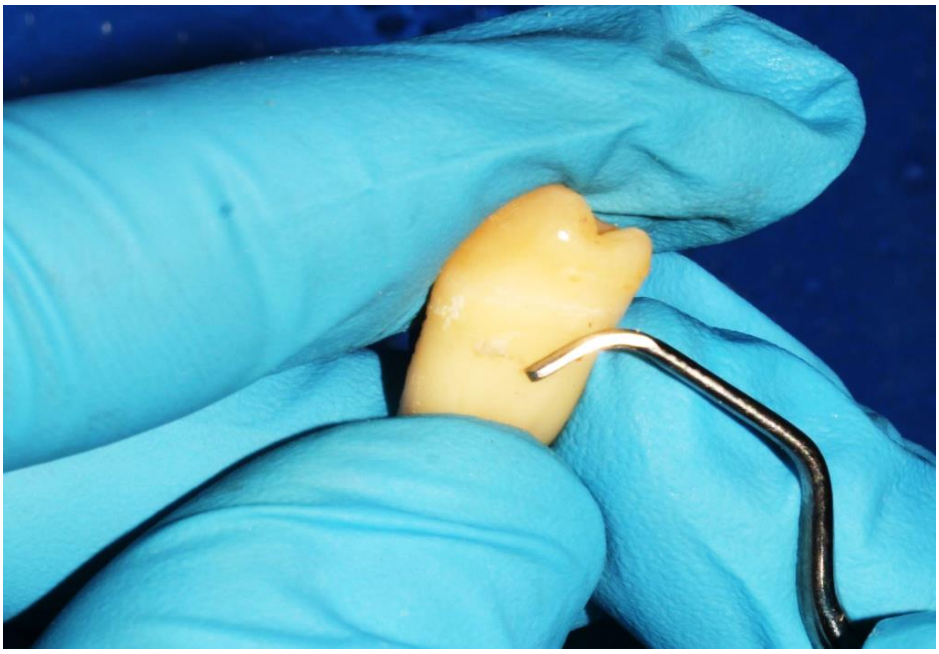

Fig. (2): a photograph showing curettage of the periodontal tissue from the external surface of the tooth.

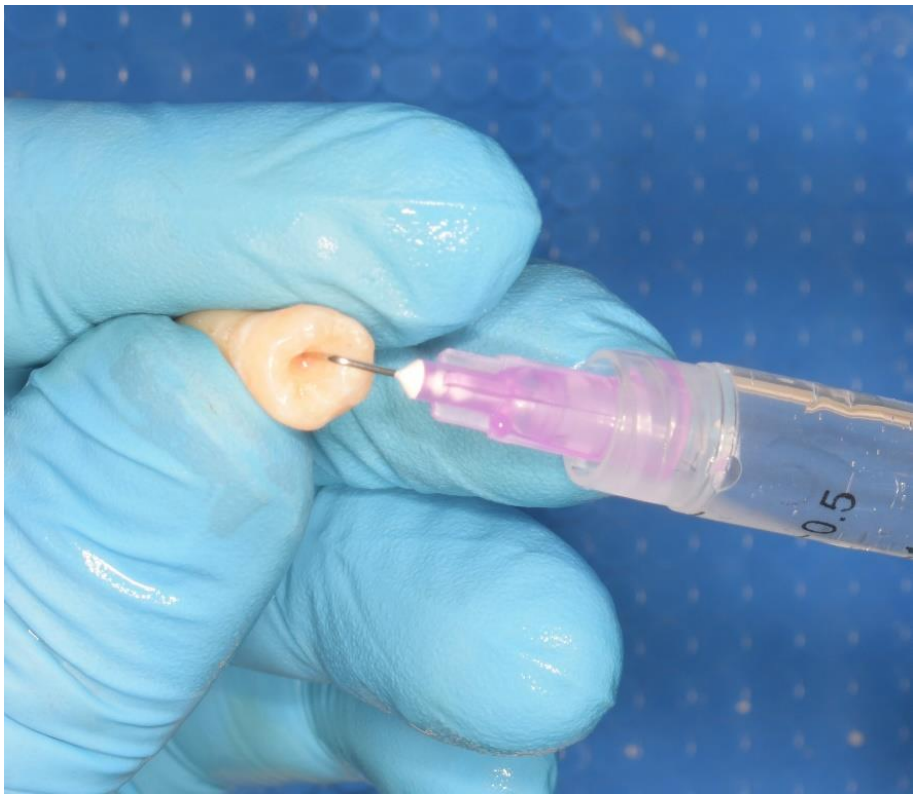

Fig. (3): a photograph showing access cavity and formalin injection in the root canal for pulp tissue fixation.

4.2 Selection and preparation of the teeth:

4.2.1. Construction of the molds and arrangement of the teeth in them.

Fabrication of five plastic molds was done to arrange the teeth inside. Each mold is 90 mm in diameter and 15 mm in thickness. Ten holes were opened in each mold with a diameter of 15 mm of each hole. Holes of each mold were filled with softened pink wax (Al-Quds company, Mansoura, Egypt) and teeth were embedded in the soft wax. For an accurate identification of the location of each tooth in the CBCT scanning, a box-shaped amalgam dot was placed at the occlusal side of the mold (fig.4). After CBCT scanning of the teeth, fifty mandibular premolars with single root canals were selected to be used in the study.

Fig. (4): a photograph showing teeth arrangement within it the plastic molds.

4.2.2. CBCT Scanning of the teeth.

A preoperative CBCT scanning of the selected teeth was done using Planmeca ProMax® 3D Plus machine (ProMax® 3D Plus, Planmeca, Helsinki, Finland) at 90 kV, 12 mA, a voxel size of 150 μm and 15 seconds exposure time to confirm presence of type I root canals according to Vertucci classification. Teeth with pulp stones, canal obliterations, internal or external root resorption, immature apex, root fractures or cracks were excluded from the study.

4.3 Grouping of the samples:

The teeth were randomly divided using Research Randomizer software (*https://www.randomizer.org*) into 5 experimental groups (n = 10) according to the size of apical preparation and whether the irrigant was activated or not:

Group 1 (n=10): root canals were prepared up to file size #30/0.04 rotary file (Hyflex CM, Coltene-Whaledent, Allstetten, Switzerland) and irrigated using CSI.

Group 2 (n=10): root canals were prepared up to size #30/0.04 Hyflex CM rotary file and the irrigating solution was activated using an ultrasonic piezoelectric device.

Group 3 (n=10): root canals were prepared up to file size #40/0.04 Hyflex CM rotary file and irrigated using CSI.

Group 4 (n=10): root canals were prepared up to file size #40/0.04 Hyflex CM rotary file and the irrigating solution was activated using an ultrasonic piezoelectric device.

Group 5 (n=10): served as negative controls which comprised un-instrumented and un-irrigated root canals.

4.4 Cleaning and instrumentation of the root canals:

Teeth with initial file size ranging from #8 to #15 K-file (Mani® stainless steel k-file, Mani inc., Togichi, Japan) were included in the study and any tooth with initial file size larger than #15 or smaller than #8 was excluded and replaced with another one. Size #10 K-file was inserted into the root canal of each sample to achieve canal patency until the tip of the file was just visible at the apical foramen (fig.5). Working length was determined by subtracting 1 mm from that length.

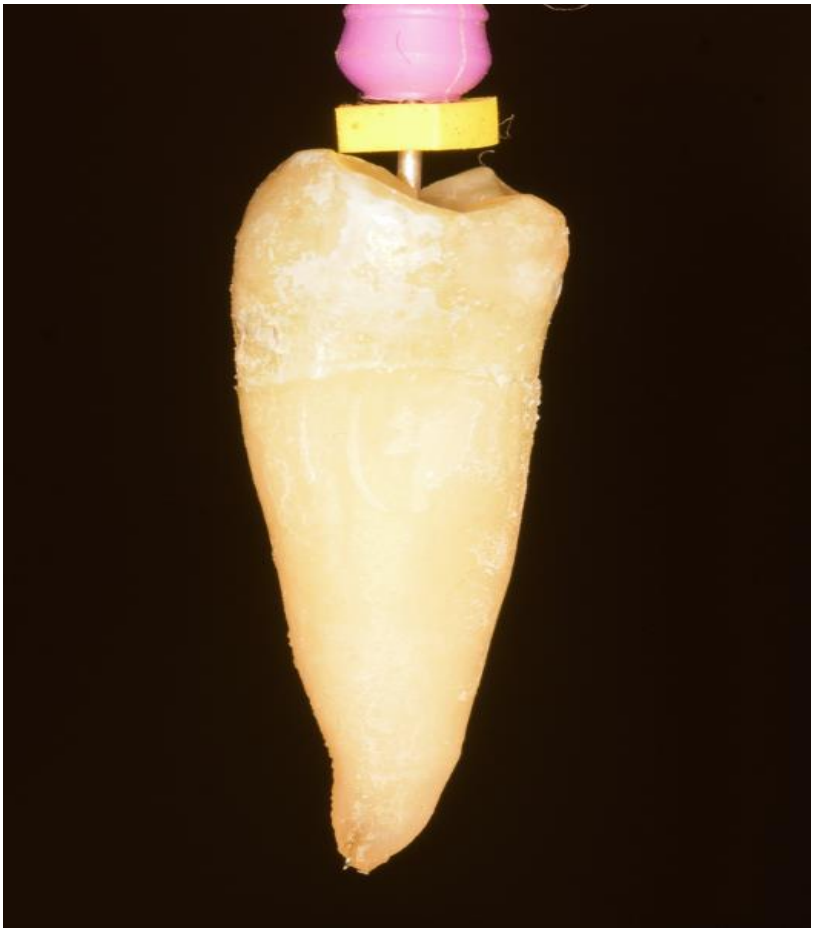

Fig. (5): a photograph showing working length determination using k-file #10.

The root canals were instrumented using Hyflex CM files which were set into rotation at the speed of 500 revolution per minute (rpm) with a rotary file handpiece powered by a cordless torque-limited electric motor (E-connect pro, Eighteeth Medical Technology Co., Changzhou, China) and the torque setting was set equivalent to 2.5 N/cm according to the manufacturer's instructions. The file size #25/0.08 was firstly used as an orifice opener and inserted slowly forward without pressure in an in-and-out pecking motion and once resistance occurred, the file was immediately removed from the canal (fig.6). File size #20/0.04 was then slowly introduced with short 1-2 mm amplitude strokes to passively insert the file to reach the full working length. File size #25/0.04 was then introduced in a pecking motion without pressure in short 1-2 mm amplitude strokes for apical enlargement followed by #20/0.06 file for shaping of middle portion of the root canal, and finally file size #30/0.04 file for further apical enlargement.

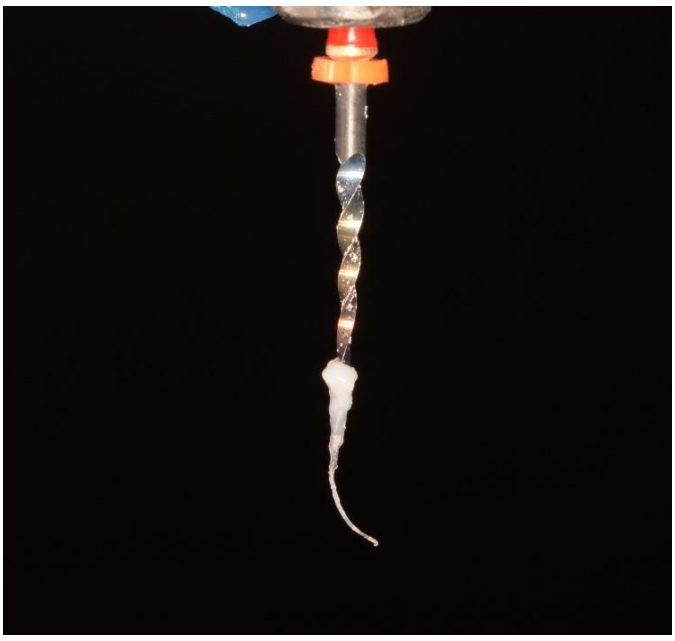

Fig. (6): a photograph showing fixed pulp tissue removed using Hyflex CM-wire orifice opener file #25/.08.

All the steps of the instrumentation procedure were carried out by a single operator. During the instrumentation procedure and in-between usage of files, apical patency was checked using manual k-file size #15/0.02 and the root canals were irrigated with NaOCl (Clorox, Egyptian company for house-hold bleach, Cairo, Egypt). The concentration and volume of the irrigating solution were standardized to 5.25% and 3 ml respectively and the irrigant delivery rate was set to 1 mL/min^{-1}[67]. The irrigating solution was delivered with a plastic syringe and a 30-gauge double side-vented irrigation needle (NaviTip needle, Ultradent Products Inc., South Jordan, UT, USA.) placed 2 mm short of the working length[68]. The canals were irrigated with 3 ml of 5.25% NaOCl, followed by 3 ml of 17% Ethylenediaminetetraacetic acid (EDTA) (MD-Cleanser™, Meta Bio-med, Chungcheongbuk-do, South Korea) then the root canals were rinsed with 3 ml normal saline (Al-mottahedoon pharma, Cairo, Egypt) as a final rinse to stop the action of EDTA[67],[69],[70].

The above-mentioned procedure was performed in all samples of Group 1 and Group 2. In Group 3 and Group 4, the samples were instrumented typically as done in Group 1 and Group 2 but with further apical enlargement of all root canals up to Hyflex CM file size 40/0.04.

After finishing the mechanical preparation in Group 2 and Group 4, PUI was carried out by inserting 3ml of 5.25% NaOCl solution inside the canal then an ultrasonic K-file size #15/0.02 attached to ED1 ultrasonic tip (ED1, DTE, Woodpecker, Guilin, China)[71] (fig.7) mounted on a piezoelectric device (DTE, D5, Woodpecker medical instrument Co., Guilin, China) was placed 2 mm short of the working length[72] and moved in an in-and-out motion for 20 seconds. The frequency of the piezoelectric device was 28 kHz and the power setting of the piezoelectric device was set to 3[73]. This process was repeated for three cycles with a total

activation time of 60 seconds and a total volume of 9 ml of NaOCl[73]. Finally, the root canals were irrigated with 3 ml of 17% EDTA then the root canals were rinsed with 3 ml normal saline as a final rinse to stop the action of EDTA.

In Group 5: neither mechanical preparation nor irrigation was performed in any sample at all.

Samples of all groups were finally dried with absorbent paper points (Absorbent Paper Points, Meta Bio-med, Chungcheongbuk-do, South Korea) with sizes corresponding to the final rotary file size used in each group.

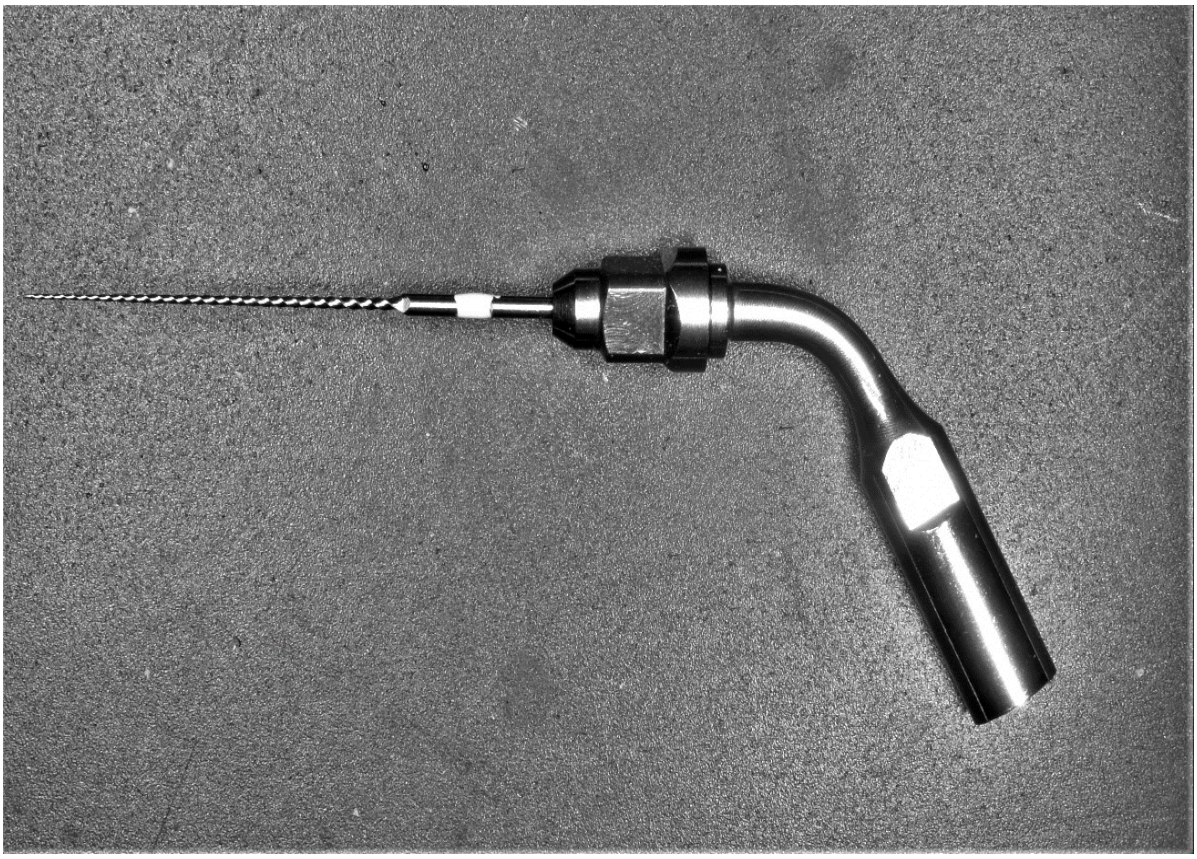

Fig. (7): a photograph showing ultrasonic K-file size #15/.02 attached to ED1 ultrasonic tip.

4.5 Histological evaluation:

Following completion of the endodontic procedures, the samples were processed for histological examination. They were fixed in 10% buffered formalin for 48h, washed in water and demineralized in a mixture of 10 wt% hydrochloric acid and 5 wt% EDTA for 1–2 weeks. The samples were rinsed with water, dehydrated, embedded in Parrafin wax, then each sample was longitudinally sectioned into two equal halves to inspect the whole length of the canal space[74]. The sections were mounted on glass slides and stained with Hematoxylin & Eosin stain. Sections of each sample were visualized using a digital microscope with an attached digital camera. The captured images were processed and the presence of pulp tissue remnants were inspected (fig.8). Each third of the root canal was given a score of 0 or 1 which indicates the absence or presence of pulp tissue remnants in the canal.

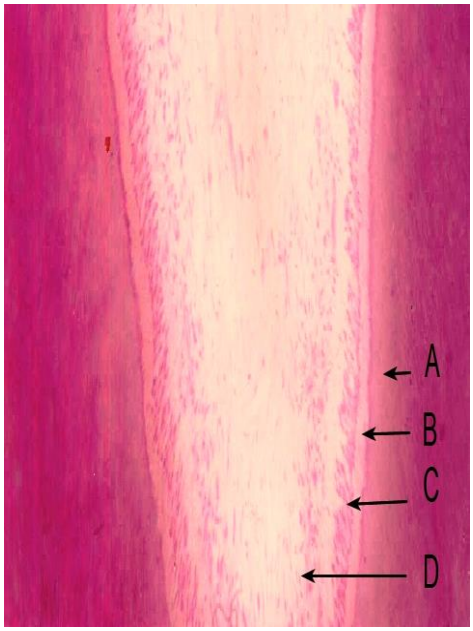

Fig. (8): The histological section at the apical part, showing (A) dentin, (B) predentin, (C) odontoblasts, and (D) connective tissue (H&E stain magnification X 32).

4.6. Statistical analysis of the data:

Qualitative data were presented as frequencies and percentages. Fisher's Exact test were used to compare between the groups. Friedman's test was used to compare between different root levels within each group. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

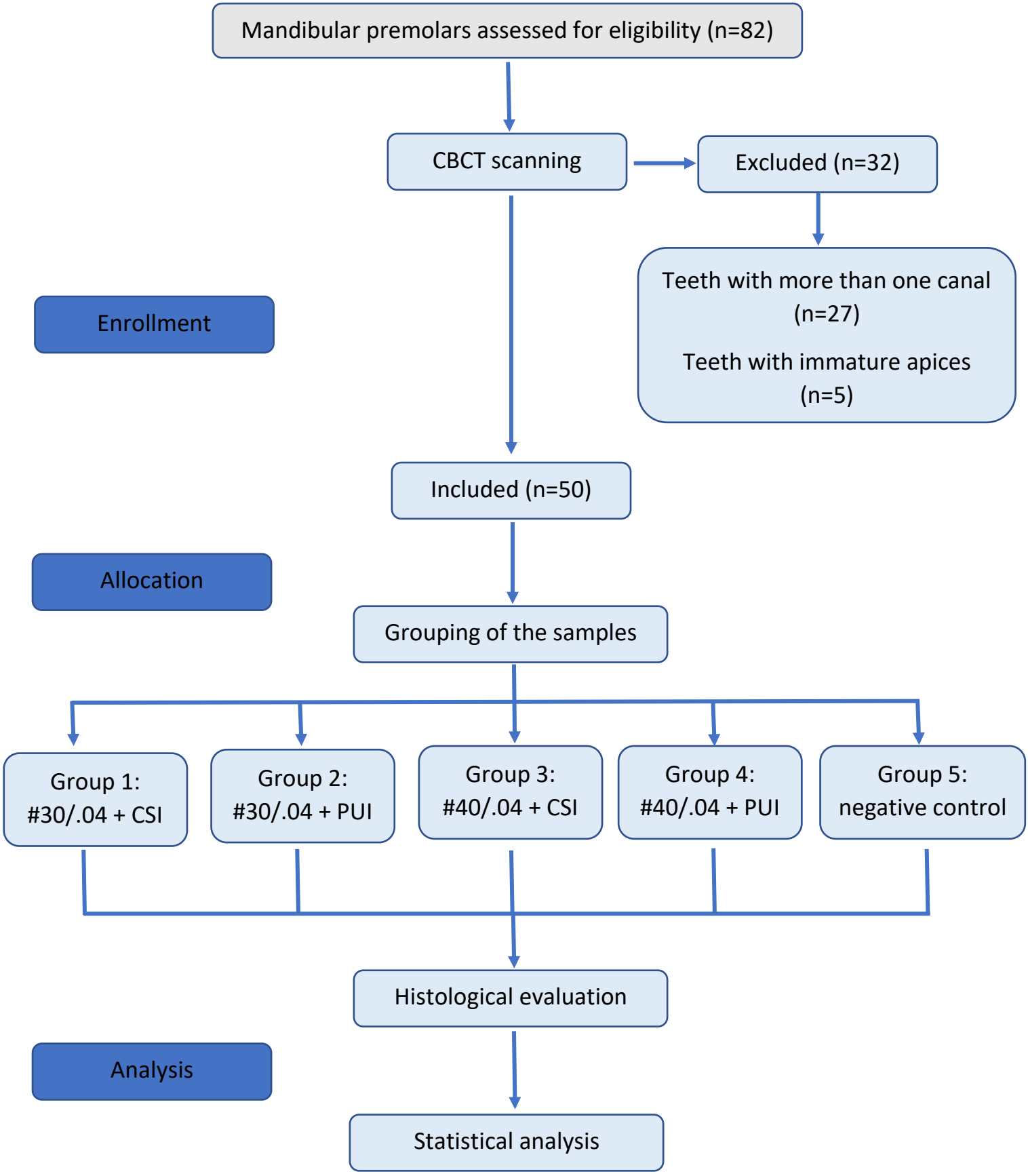

Fig. (9): A flowchart representing a review of materials and methods used in the study.

5. Results

5.1. Comparison among the tested groups.

5.2. Comparison among root levels within each group.

5.3. Descriptive histological evaluation of the groups.

5. Results

5.1. Comparison among the tested groups:

At the coronal root level, none of the specimens had pulp remnants, so no statistical comparison was performed.

At the middle and apical levels, there was no statistically significant difference between prevalence of remnants in the four tested groups (*P*-value = 1, Effect size = 0.277) for each level, respectively as shown in table no.1.

Root level	Group 1 (n = 10)		Group 2 (n = 10)		Group 3 (n = 10)		Group 4 (n = 10)		*P*-value	*Effect size (v)*
	n	%	n	%	n	%	N	%		
Coronal	0	0	0	0	0	0	0	0	Not computed	
Middle	1	10	0	0	0	0	0	0	1	0.277
Apical	1	10	0	0	0	0	0	0	1	0.277

Table no. (1): showing frequencies (n), percentages (%) and results of Fisher's Exact test for comparing among the percentage of pulp tissue remnants (%) of the tested groups**.** **: Significant at $P \leq 0.05$*

5.2. Comparison among root levels within each group:

In Group 1 (#30/.04 + conventional syringe irrigation, there was no statistically significant difference between percentage of pulp tissue remnants at different root levels (*P*-value = 0.368, Effect size = 0.1).

In Groups 2, 3 and 4, there was no pulp remnants at any root level, so no statistical comparison was performed (table no.2).

Root level	Group I (n = 10)		Group II (n = 10)		Group III (n = 10)		Group IV (n = 10)	
	n	%	n	%	n	%	n	%
Coronal	0	0	0	0	0	0	0	0
Middle	1	10	0	0	0	0	0	0
Apical	1	10	0	0	0	0	0	0
P-value	0.368		Not computed		Not computed		Not computed	
Effect size (w)	0.1							

Table no. (2): Frequencies (n), percentages (%) and results of Friedman's test for comparison between percentages of pulp remnants (%) at different root levels within each group. **: Significant at $P \leq 0.05$*

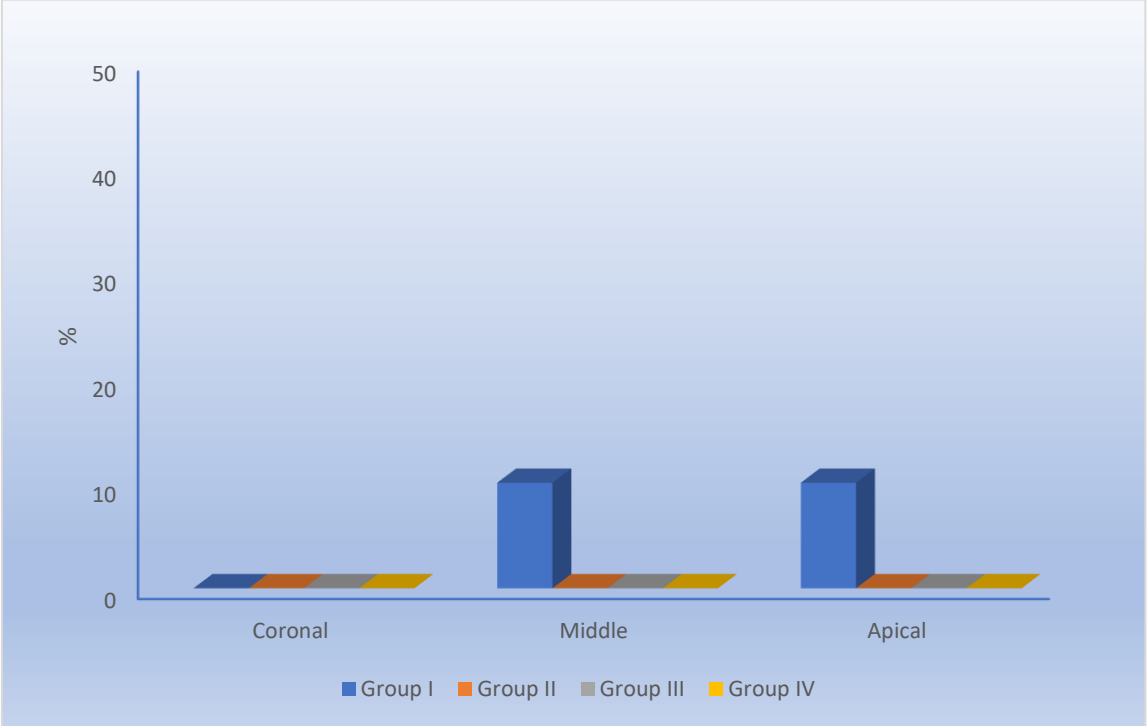

Fig. (10): Bar chart representing prevalence of remnants in the four groups.

5.3. Descriptive evaluation of the results:

- **Group1: MAF size #30/.04 with CSI:**

The microscopic images of group 1 (fig. 11 and fig.12) show irregularities in the dentinal walls along the whole length of the canals with variable depth, which reveals that all walls are touched, but remnants of pulp tissue (loose connective tissue) were still present mainly at the apical third of the root canal.

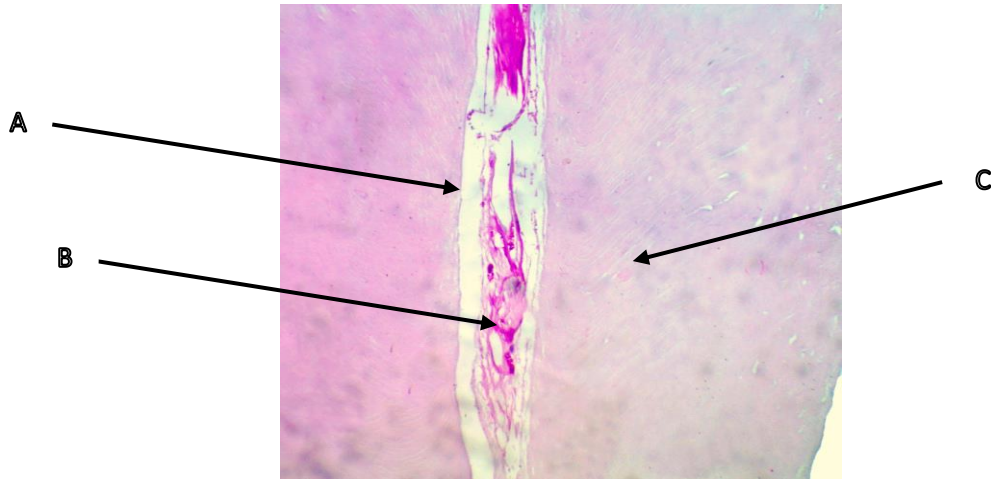

Fig. (11): photomicrograph of group 1 showing (A) irregularities along the dentinal walls, (B) loose connective tissue (pulp remnant) and (C) dentinal tubules.

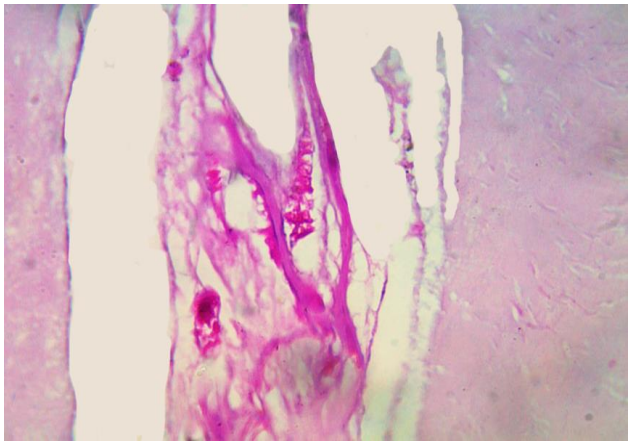

Fig. (12): photomicrograph of group 1 showing higher magnification of the previous figure.

- **Group 2: MAF size #30/.04 with PUI:**

The microscopic images of group 2 (fig. 13 and fig. 14) show irregularities in the dentinal walls along the whole length of the canals with variable depth, that reveals all walls are touched with complete absence of debris or remnants of pulp tissue.

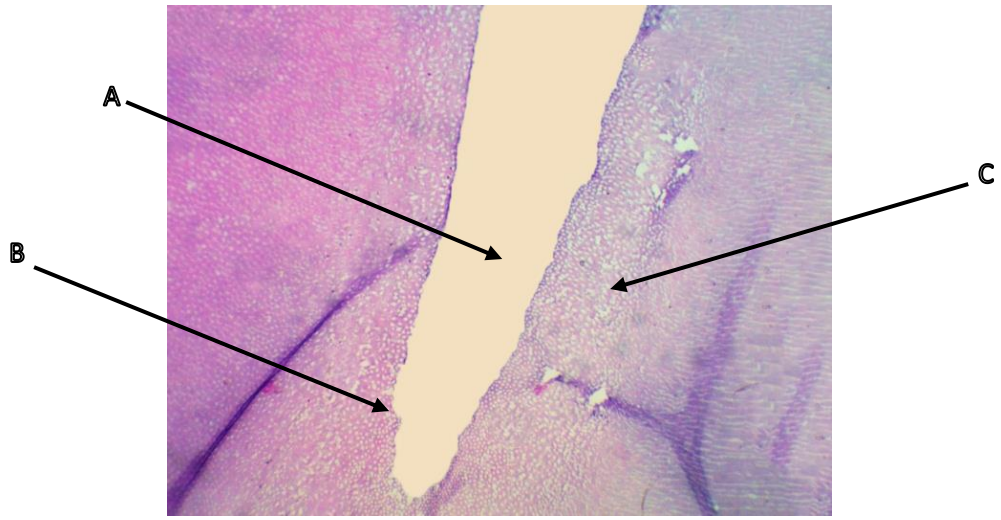

Fig. (13): photomicrograph of group 2 revealing (A) free pulp canal from any remnants, (B) irregularities along the dentinal walls, and (C) dentinal tubules.

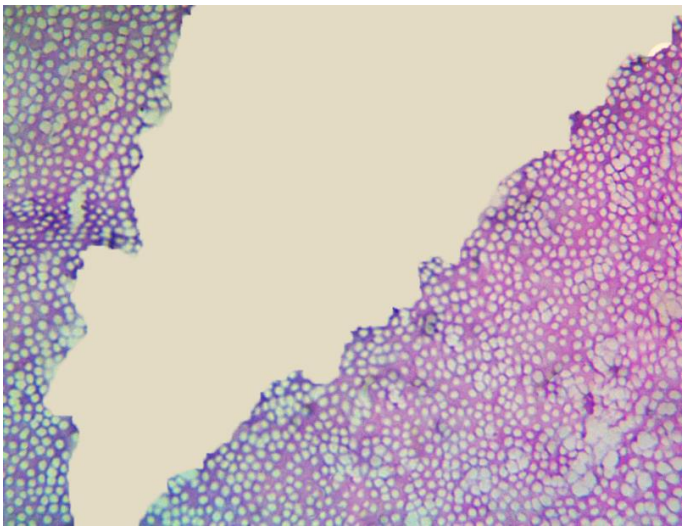

Fig. (14): photomicrograph of group 2 showing higher magnification of the previous figure.

- **Group 3: MAF size #40/.04 with CSI:**

The microscopic images of group 3 (fig. 15 and fig. 16) show irregularities in the dentinal walls along the whole length of the canals with variable depth, which reveals that all walls are touched, but at some specimens appeared with more depth compared to the previous groups and this observation appeared obviously at higher magnifications especially at the apical constriction. It shows also a complete absence of debris or remnants of pulp tissue compared to group 1 (#30/.04 with CSI).

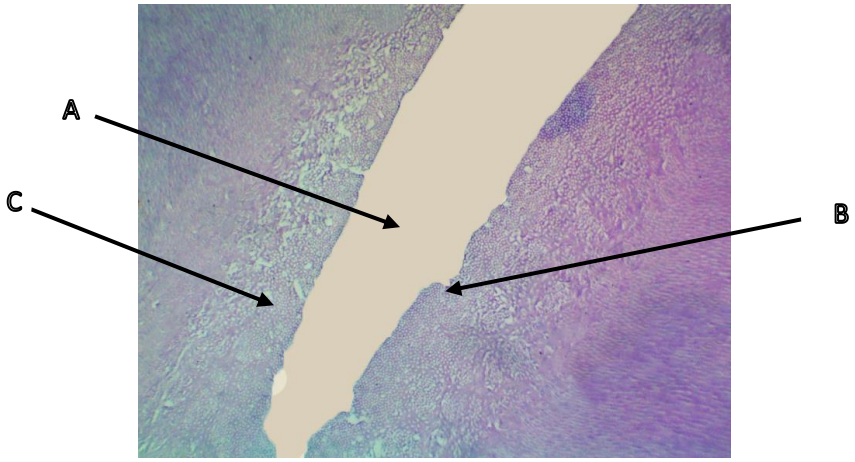

Fig. (15): photomicrograph of group 3 showing (A) free pulp canal from any remnants, (B) irregularities along the dentinal walls, and (C) dentinal tubules.

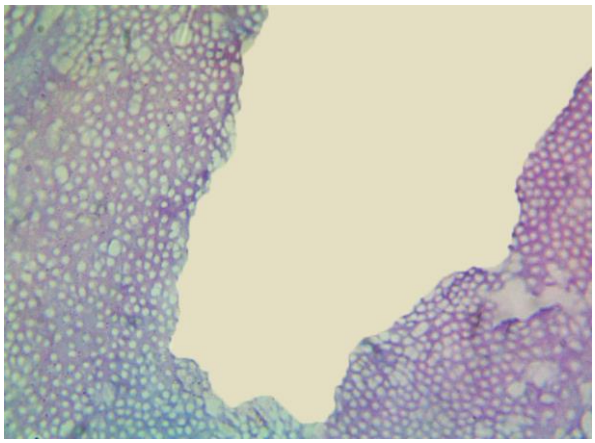

Fig. (16): photomicrograph of group 3 showing higher magnification of the previous figure.

- **Group 4: MAF size #40/.04 with PUI:**

The microscopic images of this group (fig.17 and fig.18) show irregularities in the dentinal walls along the whole length of the canals with variable depth, that reveals all walls are touched. There was also a complete absence of debris or remnants of pulp tissue.

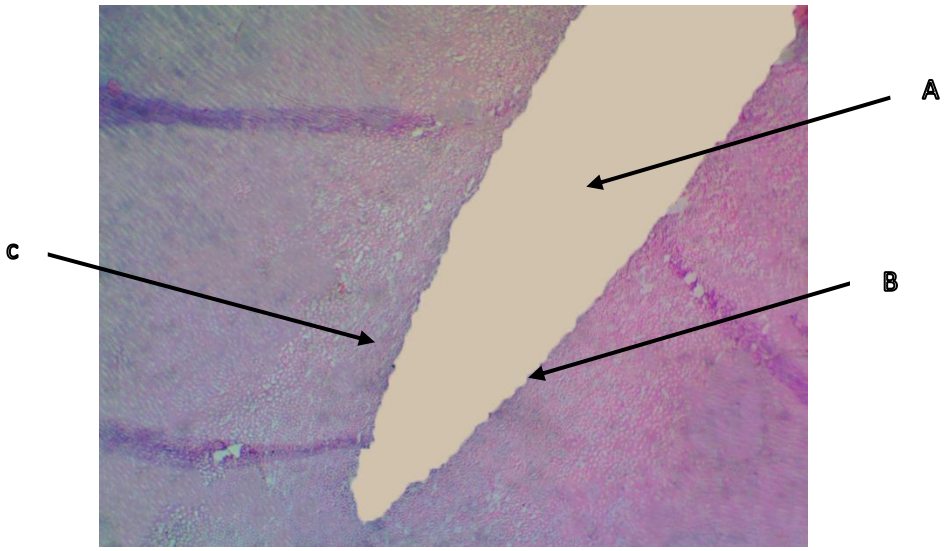

Fig. (17): photomicrograph of group 4 revealed (A) free pulp canal from any remnants, (B) irregularities along the dentinal walls, and (C) dentinal tubules.

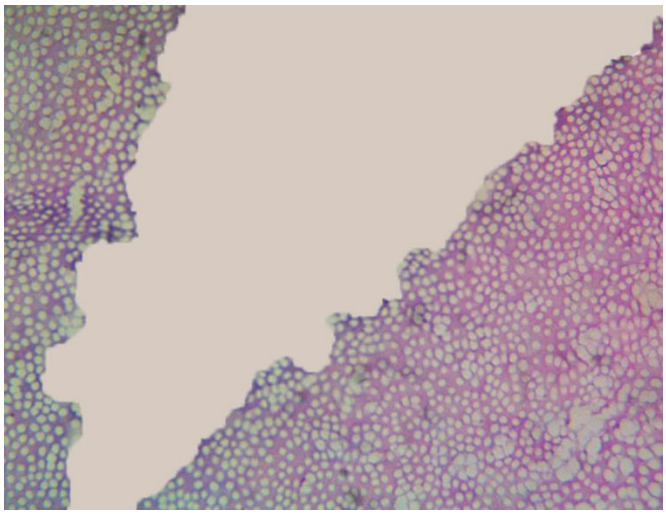

Fig. (18): photomicrograph of group 4 showing higher magnification of the previous figure.

- **Group 5: negative control group**

The histological sections of group 5, at the coronal part, revealed pulp tissue elements surrounded with dentin. The latter consists of dentinal tubules followed by predentin, then peripherally: odontoblasts outlined the pulp tissue proper. Pulp tissue proper was seen to be composed of a loosely fibrillar connective tissue stroma. In addition to the aforementioned elements, the middle and apical parts were found to be composed of connective tissue fibers with varying degrees of density, cellular elements and blood vessels (fig 19 and fig. 20).

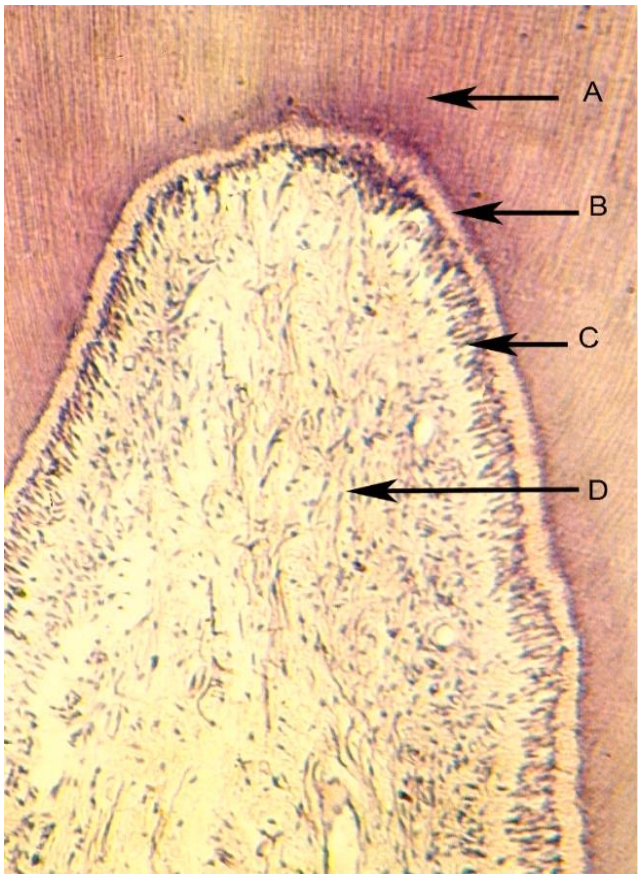

Fig. (19): photomicrograph of group 5, at the apical part, showing: dentin consists of (A) dentinal tubules, (B) predentin, (C) odontoblasts, and (D) a loosely fibrillar connective tissue (H&E stain magnification X 32)

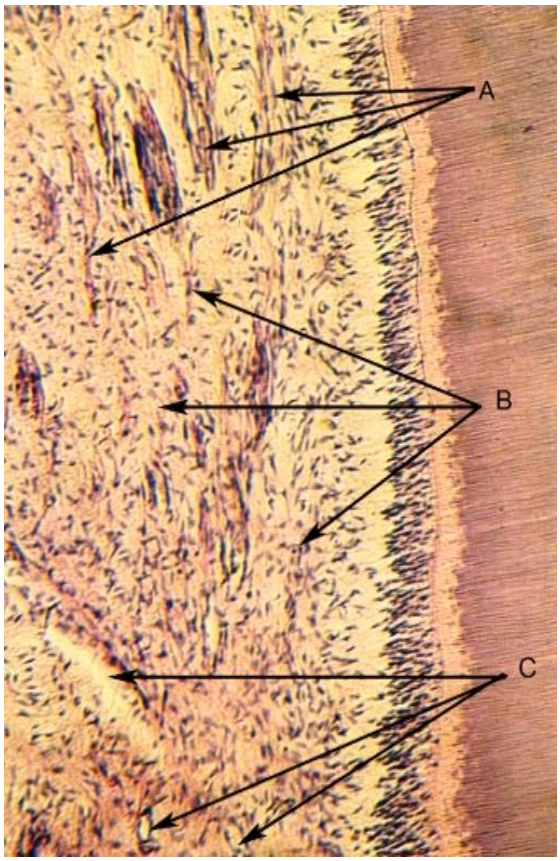

Fig. (20): photomicrograph of group 5 (control), at the middle part, showing: (A) connective tissue fibers with varying degree of density, (B) cellular elements, and (C) blood vessels (H&E stain X 100).

6. Discussion

Elimination of the pulp tissue, smear layer and microorganisms from the root canal is the main objective of the chemo-mechanical preparation[75]. Determination of the suitable master apical file size which can efficiently clean the root canal without weakening the root is controversial[76]. Moreover, the most effective irrigation protocol which can disinfect the root canal space and minimize the usage of endodontic files is still unclear[77]. This experimental, randomized, controlled, interventional prospective in-vitro study investigated the optimal MAF size and the role of irrigant activation in cleaning the root canal conservatively.

Mandibular premolars were chosen to be included in this study due to their single root and root canal configuration with availability of their extraction through orthodontic treatment[78,79]. Age range of the patients was between 18 and 40 to minimize the differences between samples regarding dentin nature and size of the pulp space[80,81].

Out of eighty-two mandibular premolars, fifty teeth were chosen to be included in this study. Twenty-seven teeth were excluded because they had more than one canal in their root canal system, and five teeth were excluded because they had immature apices.

Vitality testing was performed before extraction using pulse oximeter to ensure that pulps of all teeth are vital and healthy and any tooth with oxygen saturation level less than 90% was excluded as previous research considered this an indication of degenerative changes of the pulp tissue [82,83].

After extraction, teeth were immediately immersed in 10% neutral buffered formalin for 48 hours and then stored in 70% ethanol. This period was advised by Canene-Adams et al. for ideal preservation of the pulp tissue in its original state and to avoid any tissue hardness[66].

CBCT scanning was done to ensure that all teeth have type I root canal system according to Vertucci's classification, and to to exclude any tooth with open apex or internal root resorption or other [84–86].

Mechanical preparation of the root canals was done using Hyflex CM rotary files because they are highly flexible, heat treated, and made of a controlled memory alloy that makes the instrument exhibit superior ability to respect the internal canal morphology and produce fewer procedural errors[87–89]. Initial file size was chosen to be between #8 and #15 for standardization purposes to ensure that all the samples have close apical diameters[90].

Master apical file sizes #30/.04 and #40/.04 were chosen in this study because, despite of the abundant research work done to determine the optimal MAF size, this is still a debatable point that has not been resolved yet. Some researchers recommend that mechanical preparation up to file size #30 is sufficient[16,19,28,34,40], whilst other researchers argue that this size is insufficient and a larger instrument is needed to be used[15,26,29,30].

The irrigating solutions used were: NaOCl, EDTA and distilled water. NaOCl was used as the main irrigant during the preparation procedure because of its potent antimicrobial activity, ability to dissolve the organic content and eliminate bacterial endotoxins. The concentration of the irrigating solution was 5.25% because this concentration has higher

bactericidal action[91] and it is the most commonly used concentration level amongst dentists[8]. EDTA was used for its ability to dissolve the inorganic substances and smear layer[92–94]. The distilled water was used as a final rinse because of its isotonic properties and to stop the action of EDTA[95,96].

The irrigation protocols used were conventional syringe and double side-vented needle, and passive ultrasonic irrigation protocols. In the conventional syringe and needle protocol, it is desirable to control the irrigant delivery by rate and pressure throughout the study[97,98]. The average rate of irrigant delivery in this study was set at 1 mL/min with a gauge 30 double side-vented needle as it seemed clinically optimal and allowed the operator to maintain a constant force to avoid apical extrusion of the irrigating solution[99]. A double side-vented irrigation needle was inserted 2 mm shorter than the working length to avoid irrigant extrusion beyond the apex in order to mimic the clinical condition[68,100]. On the other hand, PUI was used to increase the irrigant efficiency through the mechanism of acoustic streaming and cavitation which creates subsequent shockwaves of irrigation and apply shear stress on the canal walls leading to dislodgement of any attached debris[9,101,102].

In this study, histological assessment was used as it is a common and reliable methodology for evaluating the amount of soft tissue remnants in the root canal[103]. Longitudinal sectioning of the samples was done to allow a clear inspection of pulp tissue remnants all over the whole length of the root[104].

When comparing different apical enlargement sizes regardless of the irrigation protocol used, both master apical files #30/.04 or #40/.04 have no detected pulp remnants in the root canals. This indicates that

apical enlargement up to file size #30/.04 is optimal and there is no need for further apical enlargement up to master apical file size #40/.04 as this may unnecessarily reduce the remaining dentin thickness and increase the liability of apical transportation and root fracture. This result is in accordance with the results of **Borges et al.**[105] in 2011 who found that apical enlargement up to file size #30 was as efficient as #45 in pulp tissue remnants elimination, and favorably resulted in less apical debris extrusion than file #45. This result was also coinciding with **Sowmya et al.**[106] in 2014 who concluded that enlarging the apical preparation size by increasing the taper of file #30 had no impact on the amount of pulp tissue remnants. Similarly, this finding is in harmony with the findings of **Lacerda et al.**[107] in 2017 who found that when root canals were prepared to size 35, .04 taper with the self-adjusting file 2.0 instrument, size 30, .04 XP-Endo Shaper or size #30/.06 TRUShape file, they revealed that the unprepared walls were less in the SAF prepared canals, however the pulp tissue debridement efficacy of the three systems was similar. This finding is also in agreement with the findings of **Alcota et al.**[64] in 2021 who examined the effect of apical enlargement on root canal debridement using histological assessment and revealed that both apical preparation up to master apical file #25 and #35 efficiently debrided the root canal with no noticeable difference between them when passive ultrasonic irrigation was applied, while when conventional irrigation protocol was used in the same study, it was found that file #35 had resulted in reduced amount of pulp tissue remnants compared with file #25. This conflict may be because file #25 is too small to efficiently debride the root canal alone without the aid of irrigant activation protocol.

On the contrary, this finding is in disagreement with the results of the in-vitro study of **Baratto-Filho et al.**[108] in 2009 who revealed that the larger the Protaper finishing file size, the lesser the pulp tissue remnants and the cleaner the root canal. This disagreement may be attributed to the difference in the instrument design and the lower concentration of the sodium hypochlorite solution used in that study. **Fornari et al.**[109] in 2010 also found that apical preparation up to file size #30/.02 and 35/.02 resulted in a significantly higher amount of pulp tissue remnants than apical enlargement up to file size #40/.02. This disagreement may be attributed to the difference in the taper of the instrument used which was .02 taper not .04 taper used in the present study and this reduction in the taper of the instrument may have resulted in poorly debrided roots canals. Another possible reason for this disagreement was that **Fornari et al.** used only distilled water as an irrigant during the instrumentation process without using sodium hypochlorite. Another study performed by **Fornari et al.**[63] in 2020 revealed that apical enlargement up to file #45/.02 resulted in lesser areas of untouched walls and reduced amount of pulp remnants than file #40/.06, these discrepancies may be attributed to the difference in tooth type, instrument design or because the range of the patients was not standardized. Also, the result of the present study is in conflict with the result of **Stringheta et al.**[23] in 2021 where apical preparation to master apical file size #30 has left more pulp remnants than file size #40. This conflict may be due to the difference in the tooth type included or because the taper of the instruments was not standardized in that study.

While when comparing different irrigation protocols with the same apical preparation size, the results of the present study showed that passive

ultrasonic irrigation had no superior efficiency over conventional irrigation protocol. This result is in agreement with **Adcock et al.**[110] in 2011 who revealed that using passive ultrasonic irrigation has no significant advantage over conventional irrigation in pulp tissue remnants removal when root canals were enlarged to file size #40/.04. This result is also in harmony with the results of **Yoo et al.**[54] in 2013 that stated that both conventional irrigation and passive ultrasonic irrigation had the same effect on organic tissue removal after root canal preparation up to file size #35/.06. The finding of the present study is also in accordance with the result of the in-vitro study of **Varela et al.**[5] in 2019 that concluded that, in round canals, conventional syringe hand irrigation and manual dynamic irrigation performed as effectively as ultrasonic irrigation in removing pulp tissue. The result of the present study is also convergent with the result of the in-vitro study of **Bago et al.**[111] in 2022 that found that conventional syringe irrigation and passive ultrasonic irrigation had comparable efficiency in removing pulp tissue remnants in the apical portion of root canals with round configuration after instrumentation with Reciproc Blue file #25/.06.

Nevertheless, the result of present study is in conflict with the results of **Boff et al.**[112] in 2014 and **Neelakantan et al.**[58] in 2016 that revealed that passive ultrasonic irrigation protocol significantly boosted the ability of the irrigating solution in pulp tissue dissolution and resulted in cleaner canals. This disagreement may be attributed to the difference in the tooth type used as **Boff et al.** used lower incisors and **Neelakantan et al.** used mandibular molars. Another possible cause for this disagreement is the lower concentration of the sodium hypochlorite irrigating solution used in both studies compared to the present study[113]. The result of **Ozlek**

et al.[65] in 2023 was in disagreement with the result of the present study as it revealed that conventional syringe irrigation left behind greater amount of pulp tissue remnants compared to the passive ultrasonic irrigation. This disagreement may be due to the difference in the total volume of the irrigant solution used throughout the instrumentation procedure as well as the difference in the file design used in that study.

Based on the results of this in-vitro study, the null hypothesis is accepted that there is no difference between the tested groups.

7. Conclusions

1- Apical enlargement up to file size #30/.04 is acceptable for effective pulp tissue debridement and there is no need for further enlargement.
2- Both of passive ultrasonic irrigation and conventional syringe irrigation protocols are effective in pulp tissue debridement.
3- Passive ultrasonic irrigation has no significant effect on pulp tissue debridement over conventional syringe irrigation.

8. Recommendations

1- Further research should be done to compare more apical sizes with different tapers.
2- Further research should be done to evaluate the effect of different irrigant activation protocols on pulp tissue debridement.
3- Further research should be done to evaluate the effect of apical enlargement and irrigant activation on pulp tissue debridement in teeth with curved roots.

9-Summary

The chemo-mechanical debridement of radicular canals to clinically satisfactory levels is of paramount importance in the success of endodontic therapy. For accomplishing this objective, extensive cleaning and shaping of the canal ought to be done, so as to evacuate any organic or inorganic debris Neither mechanical preparation nor chemical debridement alone is likely to adequately debride the root canal, especially the apical third. The root canal should be instrumented to a certain size that facilitates mechanical removal of pulp tissue remnants and microbial biofilms. Nevertheless, this should be accomplished without iatrogenic damage or compromising the structural integrity of the root. Chemical irrigation and activation are also needed to enhance the debridement without extensive mechanical preparation of the root canal.

This study was dedicated to determine the minimal apical preparation size that will efficiently remove the radicular pulp tissue remnants and whether the passive ultrasonic irrigation will enhance the efficiency of the irrigating solutions in root canal debridement.

Fifty extracted vital mandibular premolars with single root canal were included in this study.

The teeth were divided into 4 experimental groups according to the apical preparation size and irrigation protocol and 1 negative control group as follows:

Group 1 (n=10): root canals were prepared up to file size #30/0.04 Hyflex CM rotary file and irrigated using CSI.

Group 2 (n=10): root canals were prepared up to file size #30/0.04 Hyflex CM rotary file and the irrigating solution was activated using an ultrasonic piezoelectric device.

Group 3 (n=10): root canals were prepared up to file size #40/0.04 Hyflex CM rotary file and irrigated using CSI.

Group 4 (n=10): root canals were prepared up to file size #40/0.04 Hyflex CM rotary file and the irrigating solution was activated using an ultrasonic piezoelectric device.

Group 5 (n=10): served as negative controls which comprised un-instrumented and un-irrigated root canals.

Following cleaning and shaping of the samples, they were processed for histological analysis and the amount of the remaining pulp tissue was evaluated. The collected data was statistically analyzed and revealed that neither mechanical enlargement to file #40/.04 nor passive ultrasonic activation significantly improved the debridement efficiency.

Therefore, the study suggests that there may not be a significant benefit in increasing the apical preparation size beyond #30/0.04 or using passive ultrasonic activation for irrigating solutions in root canal debridement.

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الملخص العربي

إن التنظيف الكيميائي والميكانيكي للقنوات الجذرية إلى مستويات مرضية سريريًا له أهمية قصوى في نجاح العلاج اللبّيّ. ولتحقيق هذا الهدف، يجب إجراء تنظيف وتشكيل واسع النطاق للقناة، وذلك لإزالة أي بقايا عضوية أو غير عضوية. من غير المرجح أن يؤدي التحضير الميكانيكي وحده أو الغسول منفرداً إلى تنظيف القناة العصبية بشكل كافٍ، وخاصة في الثلث الذروي من الجذر. يجب تجهيز قناة الجذر بحجم معين ليسهل الإزالة الميكانيكية لبقايا أنسجة اللب والأغشية الحيوية الميكروبية، ومع ذلك ينبغي أن يتم ذلك دون ضرر أثناء العلاج أو المساس بالسلامة الهيكلية للجذر. هناك حاجة أيضًا إلى الإرواء والتنشيط الكيميائي لتعزيز عملية التنظيف دون الحاجة إلى تحضير ميكانيكي عنيف للقناة الجذرية.

تم تخصيص هذه الدراسة لتحديد الحد الأدنى لحجم التوسيع الذروي الذي سيزيل بكفاءة بقايا أنسجة اللب الجذرية ولمعرفة ما إذا كان تنشيط الغسول بالموجات فوق الصوتية سيعزز كفاءة محاليل الإرواء في تنظيف القناة الجذرية العصبية.

تم تضمين خمسين من الضواحك السفلية الحيوية ذات قناة جذرية واحدة في هذه الدراسة. وتم تقسيم الأسنان إلى ٤ مجموعات تجريبية حسب حجم التحضير القمي وطريقة الإرواء ومجموعة مراقبة سلبية واحدة على النحو التالي:

المجموعة ١ (العدد = ١٠): تم تحضير قنوات الجذر حتى مبرد مقاس #٣٠ / ٠.٠٤ مبرد دوار Hyflex CM وريها باستخدام محلول هيبوكلوريت الصوديوم باستخدام المحقن اليدوي التقليدي.

المجموعة 2 (العدد = ١٠): تم تحضير قنوات الجذر حتى مبرد مقاس #٣٠ / ٠.٠٤ مبرد دوار Hyflex CM وتم تنشيط محلول الإرواء باستخدام جهازالموجات فوق الصوتية.

المجموعة ٣ (العدد = ١٠): تم تحضير قنوات الجذر حتى مبرد مقاس #٤٠ / ٠.٠٤ مبرد دوار Hyflex CM وتم تقديم محلول الإرواء باستخدام المحقن اليدوي التقليدي.

المجموعة ٤ (العدد = ١٠): تم تحضير قنوات الجذر حتى مبرد دوار #٤٠ / ٠.٠٤ مبرد دوار Hyflex CM وتم تنشيط محلول الإرواء باستخدام جهاز الموجات فوق الصوتية.

المجموعة ٥ (العدد = ١٠): كانت بمثابة عناصر تحكم سلبية تشتمل على قنوات جذر غير مُجهزة وغير مروية.

بعد تنظيف العينات، تمت معالجتها للتحليل النسيجي وتقييم كمية أنسجة اللب المتبقية. تم تحليل البيانات التي تم جمعها إحصائيا وكشف أنه لا التوسيع الميكانيكي للمبرد رقم #٠.٠٤/٤٠ ولا التنشيط بالموجات فوق الصوتية أدى إلى تحسين كفاءة التنظيف بشكل ملحوظ.

لذلك، تشير الدراسة إلى أنه قد لا تكون هناك فائدة كبيرة في زيادة حجم التوسيع الذروي إلى ما بعد مبرد مقاس #٣٠ / ٠.٠٤ أو استخدام التنشيط بالموجات فوق الصوتية لمحاليل الري في تنظيف قناة العصبية الجذرية.

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تأثير التوسيع الذروي وتنشيط الغسول على إزالة النسيج اللبي الجذري: دراسة هستولوجية

رسالة مقدمة كجزء من مقومات الحصول على درجة الماجستير فى علاج الجذور

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طبيب أسنان بمستشفى بنها الجامعي.

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