

Comparative evaluation of penetration depth of irrigant into dentinal tubules using four different agitation Protocols - An in vitro comparative study

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Abstract

Background: Intracanal agitation technique could promote chemomechanical debridement of root canals during endodontic treatment. However there is limited evidence for the efficacy comparing ultrasonic with sonic agitation especially in apical third of root canal.

Aims and Objectives: The purpose of this study is to compare the influence of agitation on the irrigant in the

root canal treatment using four different agitation protocols at coronal, middle and apical third of the canal.

Material and Methodology: Freshly extracted human single rooted permanent premolars with single straight canals (N=80) were prepared with Protaper rotary endodontic files till F3. Alizarin red labeled sodium hypochlorite was used for final irrigation. Specimens were randomly assigned to four groups (N=20) – Group

I (conventional syringe), Group II (k file), Group III (Endoactivator) and Group IV (passive ultrasonic agitation). After completing agitation protocols, the canals dried with paper points and cut into three 1mm thick slabs at 2, 4 and 6mm from apex. Fluorescence microscope used to assess the penetration depth of irrigating solution.

Result: Passive ultrasonic agitation displayed better penetration than endoactivator and manual agitation, at all three levels coronal, middle and apical. Endo activator was better than manual agitation. And statistically non-significant difference was found between conventional syringe and k file group at all three levels.

Conclusion: Ultrasonic agitation is more effective than sonic and manual agitation.

Keywords: Alizarine red dye, Endoactivator, Fluorescence microscope, Ultrasonic.

Introduction

A successful outcome in endodontic treatment essentially depends on cleaning and shaping, disinfection and obtaining a three dimensional obturation of the root canal system¹. Even after meticulous mechanical instrumentation, organic residue and bacteria cannot be properly removed from the complexities of root canal system^{2,3}. Due to this, use of irrigation and disinfection is imperative for desired cleaner and sterile root canal space^{4,5}. Sodium hypochlorite, considered as gold standard irrigant, and is the most widely employed solution, usually at concentrations ranging from 0.5% to 5.25%^{6,7}. The antimicrobial action of sodium hypochlorite comes from hypochlorous acid which disrupts DNA synthesis in microorganisms^{8,9,10}. There are different techniques and irrigant delivery devices proposed to improve the distribution of irrigating solution within the root canal system and to reduce the

time needed for an irrigant to be effective¹¹. Agitation is one such method which is performed by introduction of an instrument into the canal and moving it with a reciprocating, oscillating or rotating action so that the irrigant is dispersed and debris is removed from the extremities of the canal¹². There are several methods by which agitation can be achieved : 1) manual reciprocation of the instrument within a canal 2) sonic or ultrasonic activation to achieve oscillation of the instrument and 3) mechanically driven rotary instrument into the canal. Manual agitation of the irrigant may be achieved using hand files, irrigation needles, well-fitting tapered gutta - percha points or micro brushes. Automated system specifically designed for agitation of the irrigant within the root canal system includes the sonic and ultrasonic devices¹². The sonic devices available for irrigant agitation include the sonic air endo handpiece and the endoactivator system. Ultrasonic devices used for agitation produce much higher frequencies (20-40kHz) based on magnetostrictive or piezoelectric mechanism. Previous studies have compared passive ultrasonic agitation with sonic and manual agitation for irrigant penetration into dentinal tubules and found different results^{13,14}. However the present study used alizarin red fluorescent dye for irrigant penetration with comparatively large sample size to evaluate the effective method of agitation in root canal treatments. Alizarin red, a fluorescent organic compound is used in biomorphic assays for quantifying the presence of calcific deposits. Calcium present in the dentin forms a precipitate with alizarin and stains immediately. So, this dye serves a good purpose in labeling hypochlorite and assessing its penetration depth in the dentinal tubules¹⁵. So the aim of this ex-vivo study was to compare passive ultrasonic agitation with sonic and manual agitation and

determine the more effective and practical irrigation method for endodontic treatments.

Materials And Method

Eighty freshly extracted permanent human single rooted premolars with straight single canal were collected from the department of oral surgery, for the study. All the teeth were cleaned, scaled, sterilized and stored in normal saline until used for the study. To standardize the canal instrumentation, the crown of each tooth was sectioned 2mm coronal to the cement-enamel junction using diamond disk at slow speed under copious cooling. A size #10 K file was inserted into the canal until it was seen through the apical foramen.

The working length was established by reducing this length by 0.5mm. The canals were cleaned and shaped with protaper rotary endodontic files till F3. Copious irrigation with 5ml of 5.25% NaOCl was performed during instrumentation using a syringe with 30 gauge needle. Smear layer removal was achieved after irrigation with 3 ml Smear Clear solution.

The exterior part of the apical IIIrd of all the samples was covered with modeling wax to prevent the irrigant from dripping through the apical foramen. A caliberated fine medium gutta percha cone was inserted till the working length to avoid intrusion of the wax from the apex¹³.

A final rinse of each canal was performed by using 5ml of 5.25% NaOCl labeled with alizarin red using 30 gauge needle keeping the tip at 3mm short of the established working length. The samples were then divided into 4 groups on the basis of different agitation protocol during the final rinse, each having 20 samples.

Group 1 (Control group): In this group agitation was performed using conventional syringe with slight up and down motion.

Group II (K file group): In this group agitation was performed using size #10k file with 20 up and down movements.

Group III (Endoactivator group): In this group agitation was performed using endoactivator at 10,000 cpm for 20 sec.

Group IV (Ultrasonic group): In this group agitation was performed using passive ultrasonic Irrigation, Satelac, at 30000rpm for 20sec. After completing the agitation protocol, the canals were dried with paper points and cut into three 1 mm thick slabs at 2,4 and 6mm from the apex. Slabs were then bonded onto glass slides and ground with wet silicone carbides papers to approximately 40 um thick. Fluorescence microscope was used at 10X under blue spectrum of light to assess the penetration depth of the irrigating solution labeled with alizarin dye into the dentinal tubules. The following set of scores were used (Raffaele P, Vittorio Franco et al, 2009)² to measure the depth of penetration of irrigating solution with alizarin red dye-

0=no visible alizarin red

1=minor traces of alizarin red

2=traces along the entire circumference of the canal wall.

3=penetration of alizarin red<50% of dentinal tubules

4=penetration of alizarin red>50% of dentinal tubule



Fig.1: 80 extracted human permanent single rooted premolars



Fig. 2: decoronation using straight handpiece



Fig.3: canals prepared

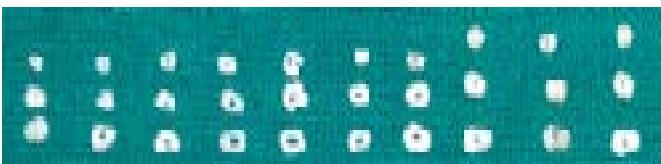


Fig. 4: transverse sectioning of samples at 2,4 and 6mm from apex Alizarin red dye penetration seen through fluorescence microscope at 2, 4 and 6mm from the apex in group I (Control group), group II (K-file group), group III (Endoactivator group) and group IV (Ultrasonic group)

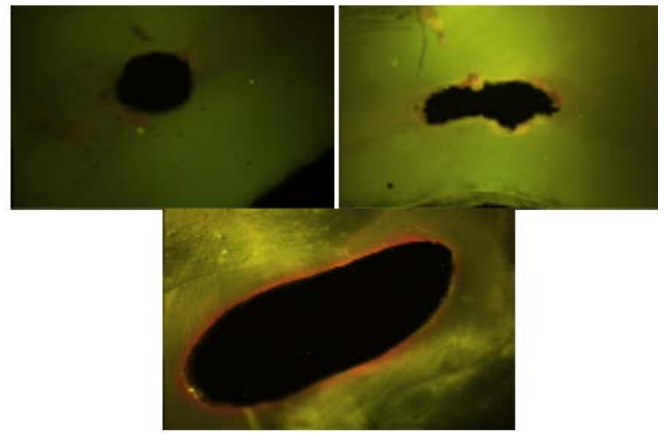


Fig 5: Alizarin red dye penetration seen through fluorescence microscope at 2, 4 and 6mm from the apex in group I (Conventional syringe group)

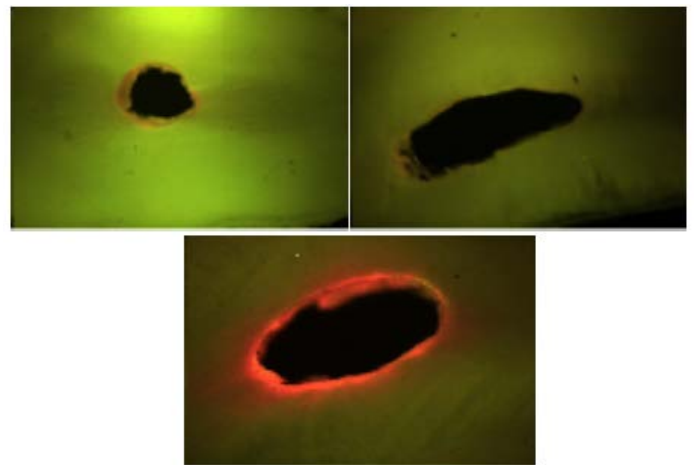


Fig. 6: Alizarin red dye penetration seen through fluorescence microscope at 2, 4 and 6mm from the apex in group II (k file group)

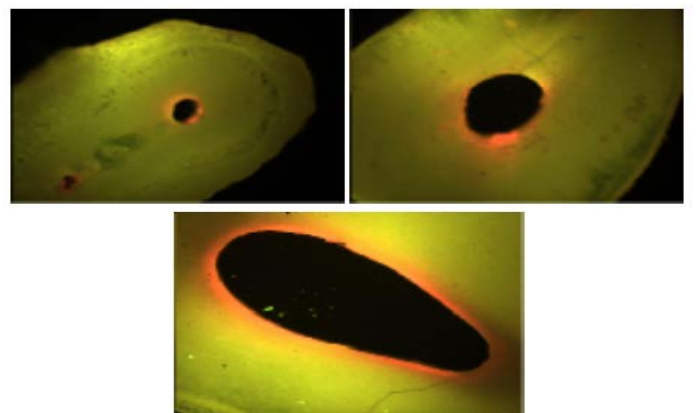


Fig. 7: Alizarin red dye penetration seen through fluorescence microscope at 2, 4 and 6mm from the apex in group III (Endoactivator group)

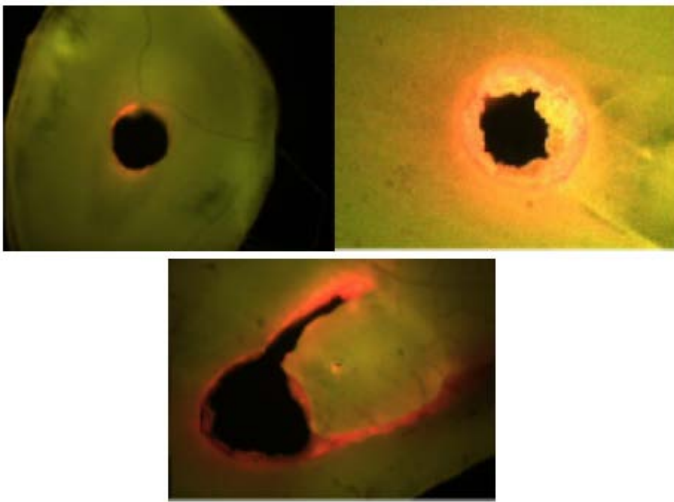


Fig. 8: Alizarin red dye penetration seen through fluorescence microscope at 2, 4 and 6mm from the apex in group IV (Ultrasonic group)

Observation and Result

Data obtained was compiled on a MS Office Excel Sheet (v2010, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Since the data is coded on an ordinal scale, non-parametric tests have been used for comparisons. Inter group comparison (>2 groups) was done using Kruskal-Wallis ANOVA followed by pair wise comparison using Mann Whitney U test. Comparison of frequencies of categories of variables with groups was done using chi square test.

- * = statistically significant difference (p<0.05)
- ** = statistically highly significant difference (p<0.01)
- # = non-significant difference (p>0.05) ... for all tables

Table 1: Group wise distribution of samples

S	Group	Description	No. of cases	Percentage
1	I	Control	20	25
2	II	K-File	20	25
3	III	Endoactivator	20	25
4	IV	Ultrasonic	20	25

Out of a total of 80 samples included in the assessment, 20 (25%) each were allocated to Groups I, II, III and IV respectively.

Group I, was the control group in which conventional syringe was used for the purpose of agitation.

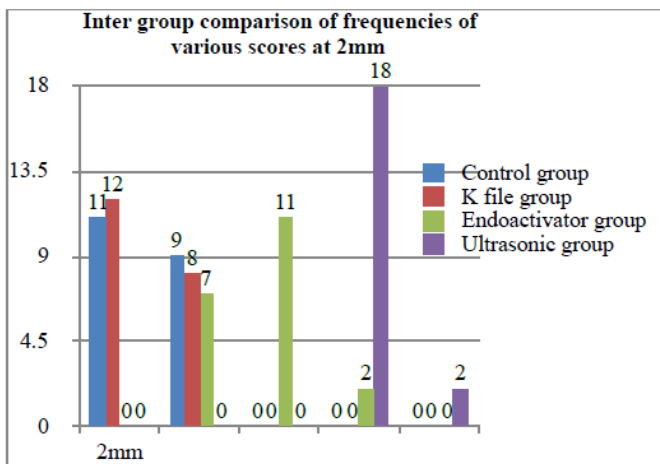
In Group II, K-files were used for the purpose of agitation. In Group III, Endoactivator was used for the purpose of agitation and finally in Group IV Ultrasonic activator was used for the purpose of agitation.

The efficacy of different agitation protocols was assessed in terms of penetration depth of the irrigant assessed using a scoring criteria at three different locations that is 2 mm, 4 mm and 6 mm from the apex. Thus in each group, a total of 60 observations were made.

Table 2: Inter group comparisons of frequencies of various scores at 2mm

	2mm					Total	Chi sq value	p value of chi sq test
	0	1	2	3	4			
Control group	11	9	0	0	0	20	116.020	0.000* *
K file group	12	8	0	0	0	20		
Endoactivator group	0	7	11	2	0	20		
Ultrasonic	0	0	0	18	2	20		
Total	23	24	11	20	2	80		

Graph 1:

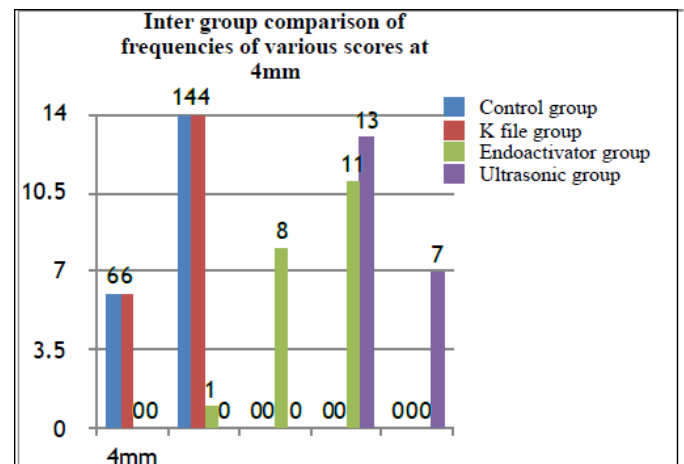


There was a statistically significant / highly significant difference seen for the frequencies between the groups ($p < 0.01, 0.05$) with higher frequency for score 0 for control group & K file, higher frequency of score 2 for Endoactivator group, higher frequency of score 3 for Ultrasonic group.

Table 3: Inter group comparison of frequencies of various scores at 4mm

	4mm					Total	Chi sq value	p value of chi sq test
	0	1	2	3	4			
Control group	6	14	0	0	0	20	106.540	0.000**
K file group	6	14	0	0	0	20		
Endoactivator group	0	1	8	11	0	20		
Ultrasonic	0	0	0	13	7	20		
Total	12	29	8	24	7	80		

Graph 2

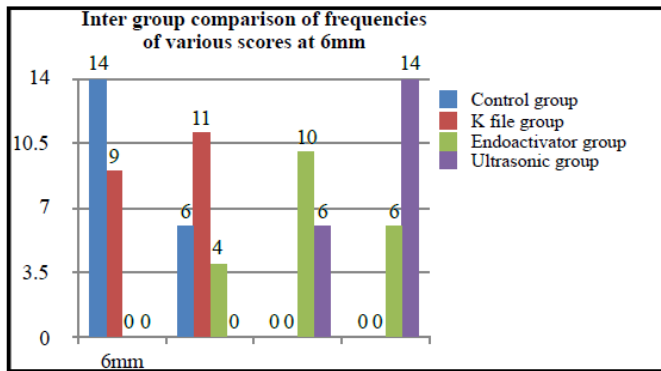


There was a statistically significant / highly significant difference seen for the frequencies between the groups ($p < 0.01, 0.05$) with higher frequency for score 1 for control group, score 2 for K file, higher frequency of score 3 for Endoactivator group, higher frequency of score 4 for Ultrasonic group.

Table 4: Inter group comparison of frequencies of various scores at 6mm

	6mm				Total	Chi square value	p value of chi square test
	1	2	3	4			
Control group	14	6	0	0	20	81.526	0.000**
K file group	9	11	0	0	20		
Endoactivator group	0	4	10	6	20		
Ultrasonic group	0	0	6	14	20		
Total	23	21	16	20	80		

Graph 3



There was a statistically significant / highly significant difference seen for the frequencies between the groups ($p < 0.01, 0.05$) with higher frequency for score 1 for control group, score 2 for K file, higher frequency of score 3 for Endoactivator group, higher frequency of score 4 for Ultrasonic group.

Table 5: Pair wise comparison using Mann-Whitney Test

Levels	Group	Vs group	Mann-Whitney U value	Z value	P value
2 mm	1	2	190.00	-0.32	0.750#
	1	3	31.500	-4.818	0.000**
	1	4	0.000	-5.777	0.000**
	2	3	28.000	-4.905	0.000**
	2	4	0.000	-5.786	0.000**
	3	4	18.000	-5.342	0.000**
4mm	1	2	200.00	0.00	1.000#
	1	3	7.000	-5.456	0.000**
	1	4	0.000	-5.657	0.000**
	2	3	7.000	-5.456	0.000**
	2	4	0.000	-5.657	0.000**
	3	4	71.500	-3.958	0.000**
6mm	1	2	150.00	-1.58	0.110#
	1	3	12.000	-5.293	0.000**
	1	4	0.000	-5.677	0.000**
	2	3	22.000	-5.027	0.000**
	2	4	0.000	-5.634	0.000**
	3	4	108.000	-2.764	0.006**

There was a statistically significant / highly significant difference seen for the values between the individual pairs of groups ($p < 0.01, 0.05$) Except for group 1 vs group 2 at each level, where there was a statistically non-significant difference seen for the values between the groups ($p > 0.05$)

Discussion

The primary goals of endodontic treatment are to debride and disinfect the root canal space and to seal it as effectively as possible aiming to establish and maintain healthy periapical tissues. Due to the intricate nature of the root canal anatomy, it is difficult to eradicate inter-radicular infection with mechanical instrumentation alone. The recently available rotary nickel titanium instruments act only on the central body of the canal,

leaving fins, culdesacs untouched after completion of biomechanical preparation." These untouched lateral canals and isthmus contains tissue remnants and biofilms. Moreover, Mc Coomb and Smith 1975)17 suggested that instrumentation produces dentinal debris which contains organic and inorganic tissue, microorganisms and their end products. This mineralized collagen matrix covering the root dentin is called the smear layer. The presence of smear layer prevents the penetration of intra-canal disinfectants and sealers into the dentinal tubules, resulting in compromised sealing ability of the root filling material and increased chances of reinfection (White et al, 1987)18. Conventional syringe agitation (Group I) showed almost equal penetration in the dentinal tubules as file activation (Group II). Although manual dynamic agitation has been advocated as a method of canal agitation as a result of simplicity and cost effectiveness, the laborious nature of this hand activated procedure hinders its application in routine clinical practice11. Group III was agitated using Endoactivator device at a frequency of 10KHz. The results of the study showed that the scores ranged from 2-3. The penetration depth of the irrigant was greater than manual dynamic agitation and conventional syringe activation. This is due to the fact that the endoactivator produces vigorous intra-canal fluid agitation through acoustic streaming and cavitation. It is used in our study at a frequency of 10 kHz which was reported to be able to effectively clean debris from lateral canals, remove the smear layer, and dislodge clumps of simulated biofilm. It is intended to provide a safer, better and faster method to disinfect a root canal system compared to other currently available conventional methods. In general, 10,000 cycles per minute (cpm) has been shown to optimize debridement and promote disruption of the smear layer and biofilm. Mancini M, Cerroni L, Lorio L

(2013)19 compared the effectiveness of irrigation with conventional syringe irrigation, manual irrigation and endoactivator system in removing the smear layer at 3, 5, and 8 min from the apex of endodontic canals. They concluded that the Endoactivator system was significantly more efficient than conventional syringe group and manual agitation in removing the smear layer and cleaning the canals at 3, 5, and 8 mm from the apex. The results of Group IV (ultrasonic agitation), on the other hand showed the penetration scores ranging between 3-4, being the highest amongst all the tested groups. The penetration of the irrigant extended in more than 50% of the dentinal tubules which is much greater than sonic agitation where the penetration was less than 50%. This result could be due to the fact that ultrasonic frequency ranges from 35-40KHz which is much higher than sonic irrigation. Therefore, cavitation and acoustic microstreaming would be greater than sonic which would result in greater antibacterial capacity and better cleanliness of the canal (Srivastava et al, 2017)13. Moreover, due to higher frequency ultrasonics cause rise in the temperature of the irrigant which is otherwise lacking in sonic agitation (Mohammadi et al, 2015)21. The temperature rise enhances the efficacy of hypochlorite to dissolve the inorganic content faster. (Pitt Ford TR, Crum LA, 2008)22. According to the researches and experimental available data the apical third appears to be most difficult to clean. Therefore, this study was done by sectioning the tooth at 2, 4 and 6mm from the apex to evaluate the penetration depth especially in the apical third and compare it with the middle and coronal third.

Conclusion

Therefore, it can be concluded that activation of the irrigant with sonic and ultrasonic have showed great improvement in cleaning efficacy of irrigants and should

be considered as one of the important adjunct to irrigation during endodontic treatment. Because this study examined only dye penetration into dentinal tubules in vitro, it is understandable that the ultrasonic agitation techniques produce better results. Since the apical third appears to be the most difficult to clean, ultrasonics which produces oscillations at a higher frequency proved to irrigate and disinfect the inaccessible areas in the apical third of the canal. Passive ultrasonic agitation displayed better penetration than endoactivator and manual agitation, at all three levels coronal, middle and apical. Endoactivator was better than manual agitation. And statistically non-significant difference was found between conventional syringe and k file group at all three levels. Moreover, long term clinical trials should be performed to evaluate the effect of these agitating devices on the dentinal microstructures.

References

1. Castelluccin A. Access Cavity and Endodontic Anatomy 2000;33:189-93
2. Paragliola R, Franco V, Fabiani C et al. Final Rinse Optimization: Influence of Different Agitation Protocols. Journal Of Endodontics 2009;21:277-80
3. Gomes J E, Aurélio K G, Texeria de Moraes MM et al. Comparison of the biocompatibility of different root canal irrigants. Journal of applied oral science. 2008; 16(2): 137-144
4. Haapasalo M, Shen Y, Qian W et al. Irrigation in Endodontics; Dental Clinics of North America 2010;291-312
5. Schilder H. Cleaning and shaping the root canal. Dental Clinics of North America. 1974;18(2):269-96.
6. Clarkson RM, Moule AJ. Sodium hypochlorite and its use as an endodontic irrigant. Australian Dental Journal, 1998 43(4):250-6 DIC
7. Agrawal VS, Mahant R, Kapoor S. A Contemporary Overview of Endodontic Irrigants - A Review. Journal of Dental Applications. 2014;7(6): 105-115. ISSN: 2381-9049
8. Poggio C, Colombo M, Scribante A et al. In vitro antibacterial activity of different endodontic irrigants. Dental Traumatology 2012; 28: 205
9. Torabinejad M, Walton R.E. Endodontic Principles and Practice. St Louis, MO: Saunders Elsevier: 2009.
10. McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clinical Microbiological Review; 1999 Jan; 12(1): 147-179.
11. Li-sha Gu, Kim JR, Ling J et al. Review of Contemporary Irrigant Agitation Techniques and Devices; Journal of Endodontics ; 2009;(6)35
12. K Gulabivala, Y-L Ng, Gilbertson M. The fluid mechanics of root canal irrigation; Physiological measurement Journal: 2010);
13. Srivastava S, Kukreja M, Kharbanda S, Grover R, Paliwal A. Effect of different methods of agitation on penetration of an endodontic irrigant. Endodontology 2017;29:156-9
14. Yu Gu, Hiran Perinpanayagam, Effect of Different Agitation Techniques on the Penetration of Irrigant and Sealer into Dentinal Tubules Photomedicine and Laser Surgery 2017.35:71-77.
15. Holcomb M, Cohen AL, McCorkle DC. An evaluation of staining techniques for marking daily growth in scleractinian corals. Journal of Experimental Marine Biology and Ecology; 2013;126-131

16. Mozo S, Llena C, Forner L. Review of ultrasonic irrigation in endodontics: increasing action of irrigating solutions *Med Oral Patol Oral Cir Bucal*. 2012 May; (3): e512-e516.
17. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. *Journal of Endodontics* 1975; 1238–42.
18. White RR, Goldman M, Lin PS. The influence of the smeared layer lipon dentinal tubule penetration by endodontic filling materials. Part II. *Journal of Endodontics*, 1987 13, 369–74. 9750
19. Mancini M, Cerroni L, Iorio L, et al. Smear layer removal and canal cleanliness using different irrigation systems : field emission scanning electron microscopic evaluation in an in vitro study. *Journal of Endodontology*. 2013;39: 1456-60
20. Walmsley AD, Lumley PJ, Laird WR. Oscillatory pattern of sonically powered endodontic files. *International Endodontic Journal*, 1989;22(3): 125-132
21. Mohammadi Z,Shalavi S,Giardino L, Impact of Ultrasonic Activation on the Effectiveness of Sodium Hypochlorite: A Review *Iran Endod J*. 2015 Fall; 10(4): 216–220
22. Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: an insight into the mechanisms involved .*Journal of Endodontics*; 1987; 13:93-101.