

EVALUATION OF THE EFFECTIVENESS OF CERTAIN IRRIGATING SOLUTIONS ON PULPAL MICROORGANISMS

N. Sukumar Singh¹, Naorem Satish Kumar Singh², Ningthoukhongjam Rati Devi³

¹Associate Professor, Department of Dentistry, J. N. Institute of Medical Sciences, Imphal.

²Assistant Professor, Department of Dentistry, J. N. Institute of Medical Sciences, Imphal.

³Postgraduate Student, Department of Conservative Dentistry and Endodontics, Jaipur Dental College, Jaipur.

ABSTRACT

Successful root canal involves as thorough cleansing of the root canal with an irrigating solution. Irrigating solutions dissolve necrotic tissues and debris from crevices and in many instances to root canal instruments and acts as lubricants for root canal instruments and makes the negotiation and enlargement of the canal far easier and reduces the possibility of instrument breakage. Although many author advocate use of different irrigating solution. Less was published regarding the use of combination of 0.2% Chlorhexidine and neutral EDTA as an irrigating solution. Study was performed in 80 single rooted teeth and irrigated using 0.2% Chlorhexidine, neutral EDTA, combination of both and 0.9% normal saline which is control group at different time appointment. It was found out that the reduction percentage in the teeth treated with 0.2% chlorhexidine within the canal after 15 mins., 48 hrs. and 96 hrs. are 58.78%, 79.97% and 90.35% respectively. The reduction percentage in that of neutral 0.2% EDTA at the same interval appointments were 21.52%, 29.14% and 40.30% respectively. The reduction percentage in microorganisms treated with combination of both solution after 15 mins., 48 hours and 96 hours were 89.96%, 92.96% and 95.41% respectively. This concludes that use of combination of 0.2% chlorhexidine and neutral EDTA was found to be the most suitable irrigating solution in success of root canal treatment.

KEYWORDS

Chlorhexidine, EDTA, Irrigation, Microorganisms.

HOW TO CITE THIS ARTICLE: Singh NS, Singh NSK, Devi NR. Evaluation of the effectiveness of certain irrigating solutions on pulpal microorganisms. J. Evolution Med. Dent. Sci. 2016;5(74):5478-5483, DOI: 10.14260/jemds/2016/1240

INTRODUCTION

With the advancement of Endodontics, it has been made possible to save those teeth that were once thought to be beyond repair and have a relatively bad prognosis. The main tool regarding this that came into limelight is the root canal treatment.¹

The primary aim and objective of root canal treatment is the retention of pulp less or pulpally involved tooth with its association of periapical tissue in a healthy state.^{2,3} Achievement of this objective necessitate that the pulpal space and their contents be eliminated as source of irritation. Irritants contained in these spaces are protein degrading products and microorganisms living and growing in the decomposed tissue. They evoke inflammatory responses in adjacent tissues.^{4,5}

A thorough debridement and canal preparation procedure should be given the highest priority in the hierarchy of the root canal treatment. Mechanical instrumentation and irrigation using irrigating solutions greatly enhance the effectiveness of the preparation of the root canal obturation.⁶

Irrigating solutions dissolve necrotic tissues and debris from crevices and in many instances to root canal instruments. It also acts as lubricants for root canal instruments and makes the negotiation and enlargement of the canal far easier and reduces the possibility of instrument breakage.^{7,8}

Financial or Other, Competing Interest: None.

Submission 24-08-2016, Peer Review 06-09-2016,

Acceptance 08-09-2016, Published 15-09-2016.

Corresponding Author:

Dr. Naorem Satish Kumar Singh,

#79/10 Nagamapal Singjubung Leirak,

Imphal West-795001, Manipur.

E-mail: drnsatish@gmail.com

DOI: 10.14260/jemds/2016/1240



Clinical usefulness of bacteriological control in endodontic therapy has been demonstrated by the great success rate, which occurs in teeth filled after obtaining a negative culture.^{8,9,10} The microbial control within the pulpal tissue and the root canal space is a pre-requisite for the prevention and treatment of pulpal and periapical breakdown. Unfortunately, it is difficult to eliminate all microorganisms and organic debris from the root canal system regardless of the irrigant and instrumentation.^{11,12}

In the present study, the antibacterial effect of certain irrigating solutions have been compared in the treatment of teeth with necrotic pulp.¹³ The efficacy of the treatment has been evaluated with bacteriologic methods.

AIMS AND OBJECTIVE

The purpose of this study is to evaluate the antibacterial effect of two different irrigating solutions, i.e. 0.2% chlorhexidine and neutral EDTA at the microorganisms in pulp cavity in different time period of irrigations.

MATERIALS AND METHODS

For the study, single rooted non-vital anterior teeth with necrotic pulps and intact pulp chamber walls were the criteria for the case selection. Oral prophylaxis was done of the selected segment and 80 positive samples were taken for the study. The patients were divided into experimental group for the use of three different irrigating solution, i.e. chlorhexidine, EDTA and combination of chlorhexidine and EDTA and a control which uses normal saline. They were asked to rinse their mouth with chlorhexidine mouthwash twice or thrice daily. The tooth from which the sample was to be taken was cleaned with pumice and isolated using rubber dam.

The tooth and the clamp were cleansed with 30% hydrogen peroxide for 1 minute.^{14,15} An initial opening was

made, 1 to 2 mm into the dentin with a sterile fissure bur at high speed. The tooth was swabbed with hydrogen peroxide for 10 seconds followed by application of 2% tincture of iodine for 1 minute. The iodine was deactivated with 5% sodium thiosulphate.

For proceeding the penetration of the roof of the pulp chamber, a sterile No. 2 or No. 4 tungsten carbide round bur was used at slow speed, working the bur from the lingual surface to expose the pulp chamber.^{16,17}

After the exposure of pulpal roof, the pre-irrigant sample fluid from the canal was soaked up by absorbent paper point (Dentsply) and transferred to a bottle containing 15 mL thioglycolate (Dynamicro GR Thane West, Mumbai).¹⁶ After biomechanical preparation of the root canal for 15 minutes. The irrigating solution was introduced into the canal by means of a sterile syringe and a 27-gauge needle that was not allowed to bind to the canal walls. For every group, culture was taken in the following way. In both groups, 9 mL of irrigating solution was used by three instalments of 3 mL each. After 15 minutes, the final irrigation was done with corresponding irrigating solution and a specimen for microbiological examination was taken as post-irrigant sample.

After 48 hours and 96 hours, the patient was called for second appointment. Another specimen for microbiological examination was collected after 15 minutes of instrumentation and irrigation at the end of the second appointment.

The pre-irrigant and post-irrigant samples were streaked on separate blood agar plates. Plates were incubated at 37° C for 72 hrs. in an anaerobic chamber (Dynamicro Gaspak System). After incubation Colony Forming Units (CFU) were counted on the plate with colony counter. The organisms were identified by standard microbiological procedures.^{10,17}

OBSERVATION AND RESULTS

For the study, eighty single rooted teeth with necrotic pulp were randomly selected from patients attending dental needs at the hospital. None of the patients were on antibiotic therapy for at least three months before or during sample collection. Samples were divided into four groups of twenty teeth each categorising three experimental and one control group namely, A1 (Irrigant 0.2% chlorhexidine gluconate), A2 (Neutral 0.2% EDTA), A3 (0.2% Chlorhexidine gluconate + Neutral 0.2% EDTA) and control group B (0.9% normal saline). Strict aseptic technique was carried out throughout the study. Every tooth was treated in three appointments and four samples were collected from the same patient at the interval of pre irrigation, after 15 minutes of first irrigation, after 48 hours (Second irrigation) and after 96 hours (Third irrigation); 9 mL of desired irrigating solution was used for each appointment.

It was observed that bacterial flora (Anaerobic) present in the root canal were streptococcus viridans, enterococcus, staphylococcus albus, peptostreptococcus, bacteroides species and fusobacterium species. The microbial growth within the root canal before irrigation and after irrigation in all four groups were quantitatively analysed and studied. The results showed that the number of post irrigant positive cultures (CFU/mL) was significantly reduced in all four groups (P < 0.001) compared with the pre-irrigant positive culture using t-test.

In sub-group, all the samples show positive culture report in pre irrigation sample. Post irrigation sample of 0.2% chlorhexidine gluconate after 15 minutes shows reduction of microorganism. Here, sample taken after 48 hours showed negative culture in two samples, whereas the sample taken after 96 hours showed negative culture in four samples. The reduction percentage in the teeth treated with 0.2% chlorhexidine within the canal after 15 mins., 48 hrs. and 96 hrs. are 58.78%, 79.97% and 90.35% respectively. The reduction percentage in that of neutral 0.2% EDTA at the same interval appointments were 21.52%, 29.14% and 40.30% respectively (Table I).

In post irrigation sample of 0.2% chlorhexidine + Neutral 0.2% EDTA after 15 minutes showed reduction of microorganism, but after 48 hours five samples negative culture, whereas after 96 hours showed eight negative culture. The reduction percentage in microorganisms treated with this solution after 15 mins., 48 hours and 96 hours were 89.96%, 92.96% and 95.41% respectively (Table I).

In controlled group where samples were treated with 0.9% normal saline showed increase in microorganisms in nine samples after 48 hours (Second irrigation) and eight samples after 96 hours (Third irrigation). The reduction percentage with 0.9% normal saline at the same interval appointments were 60.84%, 65.62% and 57.55% respectively (Table I).

Group	15 mins. (First Irrigation)	48 hrs. (Second Irrigation)	96 hrs. (Third Irrigation)
A1	58.78	79.97	90.35
A2	21.52	29.14	40.30
A3	89.96	92.96	95.41
B	60.84	65.62	57.55

Table I: Intergroup Comparison in Percentage Reduction

After applying 0.2% chlorhexidine gluconate, mean and standard deviation of the value of each stages showed that there is a declining trend from pre irrigation to the respective post irrigation stages with the lowest value observed at post irrigation after 96 hours (Table II). T-test showed highly significant deduction from one stage irrigation to another stage irrigation (Table III).

Sl. No.	Irrigation Time	Sample Size	Mean	S.D.
1	I Pre (0 hr.)	20	11267600	30492168
2	II Post (15 mins.)	20	4893940	12158107
3	III Post (48 hrs.)	20	2256660	5966642
4	IV Post (96 hrs.)	20	1086400	2747354

Table II: Distribution of Mean of Subgroup A1 Irrigated by 0.2% Chlorhexidine with Respect to the Pre and Post Irrigations

Between	t-Value	d.f.	p-Value	Remark
I&II	4364.58	38	<0.001	VHS
I&III	6673.96	38	<0.001	VHS
I&IV	7897.44	38	<0.001	VHS
II&III	4903.83	38	<0.001	VHS
II&IV	4410.48	38	<0.001	VHS
III&IV	1772.91	38	<0.001	VHS

Table III: Result of T-Test of Table II

Sl. No.	Irrigation Time	Sample Size	Mean	S.D.
1	I Pre (0 hr.)	20	12826000	30101516
2	II Post (15 mins.)	20	10064915	20777194
3	III Post (48 hrs.)	20	9088545	18880982
4	IV Post (96 hrs.)	20	7881510	16365978

Table IV: Distribution of Mean S.D. of Subgroup A2 Irrigated by Neutral 0.2% EDTA with Respect to Pre and Post Irrigation

Sl. No.	Irrigation Time	Sample Size	Mean	S.D.
1	I Pre (0 hr.)	20	1183150	3035438
2	II Post (15 mins.)	20	463306	1439966
3	III Post (48 hrs.)	20	406723	1218597
4	IV Post (96 hrs.)	20	502145	1272527

Table VIII: Distribution of Mean S.D. of Sub Group B Irrigated by 0.9% Normal Saline with Respect to Pre and Post Irrigation

Between	t-Value	d.f.	p-Value	Remark
I&II	1695.69	38	<0.001	VHS
I&III	2389.43	38	<0.001	VHS
I&IV	4030.34	38	<0.001	VHS
II&III	691.51	38	<0.001	VHS
II&IV	1596.52	38	<0.001	VHS
III&IV	399.59	38	<0.001	VHS

Table V: Result of T-Test of Table III

Between	t-Value	d.f.	p-Value	Remark
I&II	1521.72	38	<0.001	VHS
I&III	1683.50	38	<0.001	VHS
I&IV	1467.33	38	<0.001	VHS
II&III	155.19	38	<0.001	VHS
II&IV	138.74	38	<0.001	VHS
III&IV	273.20	38	<0.001	VHS

Table IX: Result of T-Test of Table VII

In the case of neutral 0.2% EDTA, there is also sharp decrease in microorganisms over four different stages of irrigations with the lowest value at after 96 hours irrigation (Table III). T-test showed highly significant different population of microbial flora between the combinations of IV different irrigation stages (Table IV). Similarly, in case of irrigation used in combination of 0.2% chlorhexidine gluconate and neutral 0.2% EDTA observed sharp decrease in microbial flora from pre irrigation to different interval stages of post irrigation (Table V). Quite interestingly it is observed that in the case of control group, the number of organisms sudden fall from pre irrigation at 15 mins. after irrigation then little decrease in after 48 hrs. irrigation and later rises after 96 hrs. of post irrigation. The variation of mean values of microorganisms for 4 groups understudied with respect to 4 stages of irrigation were variably seen and can be decided using two way Variance ratio test. There is no variation of mean among groups despite visible difference, as F=1.23 for 3,9 d.f. at 5% probability is insignificant.

Group	0 hr.	15 mins.	48 hrs.	96 hrs.
A1	61776522	6046382	4395607	502145
A2	12826000	10064915	9088545	7881510
A3	11267600	4893940	2256660	1086400
B	1183150	463306	406723	502145

Table X: Distribution of Means of Organisms over the Groups

Source of Variation	S. S	d. f.	M. S. S.	F-Value	P-Value	Remarks
Due to Groups	683016318.5	3	227672106	1.23	>0.05	Insignificant
Due to Stage	966835809	3	322278603.2	1.748	>0.05	Insignificant
Error	1659328216	9	184369801.8			

Table XI: ANOVA on the Data of Table IX

Sl. No.	Irrigation Time	Sample Size	Mean	S.D.
1	I Pre (0 hr.)	20	61776522	222896976
2	II Post (15 mins.)	20	6046382	16408546
3	III Post (48 hrs.)	20	4395607	11583069
4	IV Post (96 hrs.)	20	502145	1272527

Table VI: Distribution of Mean S.D. of Sub Group A3 Irrigated by 0.2% Chlorhexidine Gluconate + Neutral 0.2% EDTA with Respect to Pre and Post Irrigation



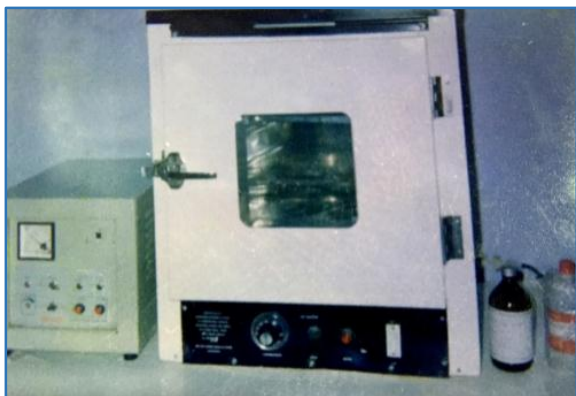
Picture 1: Different Irrigants used in the Study

Between	t-Value	d.f.	p-Value	Remark
I&II	57731.69	38	<0.001	VHS
I&III	16758.28	38	<0.001	VHS
I&IV	242918.39	38	<0.001	VHS
II&III	1395.36	38	<0.001	VHS
II&IV	5626.72	38	<0.001	VHS
III&IV	15435.38	38	<0.001	VHS

Table VII: Result of T-Test of Table VI



Picture 2: Transport Media



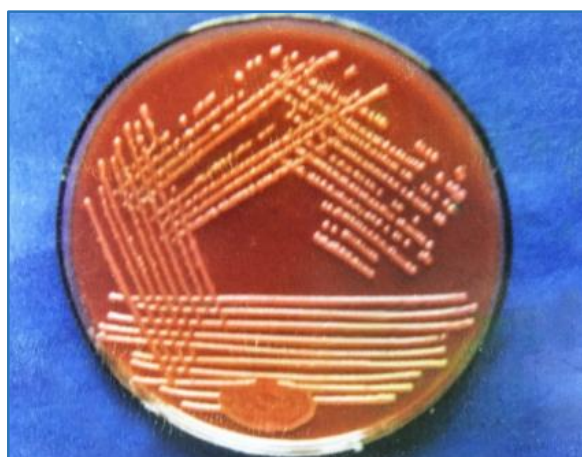
Picture 3: Incubator



Picture 4: Anaerobic Gas Tank



Picture 5: Colony Counter



Picture 6: Bacterial Colony

DISCUSSION AND CONCLUSION

To achieve the desired goal of root canal treatment, L.I. Grossman¹⁸ described three phases of treatment, i.e. biomechanical preparation, chemical preparation and sterilisation. Ingle and Zeldow¹⁷ have shown that mechanical enlargement and shaping of the root canal are the first giant step towards a clean and aseptic pulp cavity. During enlargement and shaping, irrigation of root canal is necessary to remove both hard and soft tissues by washing out and removing microorganisms to prevent pulpal and periapical infections. Studies have shown that infected root canals are mainly of anaerobic microorganisms^{11,19,17} (90%) and are the main cause of pain, swelling, sinus tract formation and halitosis.^{16,20,1,21} Various irrigants have been used in root canal therapy to reduce or eliminate bacteria, many of them have shown varying degrees of antibacterial activity. In this study, four irrigating solutions have been taken to see their efficacy on root canal viz. chlorhexidine, EDTA, chlorhexidine + EDTA and normal saline.

The result of this study indicates that chlorhexidine and EDTA combination showed greater percentage of reduction of microbial flora (95.4%) when compared with the individual use of chlorhexidine or EDTA.

Chlorhexidine was found to be more effective in its antibacterial property when compared with EDTA with a percentage reduction of 90.35% after 96 hours irrigation from first irrigation in case of chlorhexidine, while EDTA comparatively have a less percentage of reduction of 40.3% after 96 hours. The different data of mean and standard deviation for different groups at different time period showed significant reduction of microbial counts, which was confirmed with S. Patterson et al²² and Vahdaty et al²³ study of antibacterial property of chlorhexidine. Chlorhexidine, a cationic bis-guanide has optimal microbial action in between pH 5.5 to 7. It has been found to be effective against a variety of microorganisms, such as gram +ve and gram -ve bacteria, bacterial spores, lipophilic virus, yeast and dermatophytes being bacteriostatic at low concentration and bactericidal at high concentration. In addition to this, acidic protein such as hydroxyapatite absorbs chlorhexidine and releases it gradually in the form of active cation (substantivity), justifying its clinical use as a root canal irrigating solution at different concentration both in vitro and vivo.^{24,21,25}

Neutral EDTA was found to be effective against root canal flora as an irrigating solution. In our study, it was found to be statistically significant in different time period of irrigation. Nikiforuk et al²⁶ also established that EDTA being a good chelating agent causes demineralisation of hard tissue at neutral pH. On the contrary, Heling B. et al²⁷ found out to be neither bactericidal nor bacteriostatic, but it inhibits the growth of bacteria and eventually destroys bacteria by starvation. It produce no deleterious effect rather its use as an irrigant facilitated biomechanical preparation, as it removes loose debris and softened partially calcified necrotic pulp remnants. Its only drawback is that it makes radicular wall more permeable to microorganism diffusion, because of its chelating property.^{12,19} We have found in our study that EDTA as irrigating solution has no deleterious effect on the tissue, so it can be used as an irrigant.²¹

It may be further emphasised that the combination of EDTA and Chlorhexidine⁹ proved to be even better irrigant as

they suffice to the potentiality of both the irrigants. It acts synergistically by enhancing the bactericidal effect of chlorhexidine after the debridement and removal of smear layer by EDTA. It also prevents further demineralisation effect of EDTA, which is proportional to the exposure time, thereby increasing the efficacy of the said combination. It may be further emphasised that the combination of EDTA and chlorhexidine proved to be an even better irrigant as compared to EDTA and chlorhexidine individually. In our study, we have found that the combination of chlorhexidine and EDTA show reduction percentage 89.96 after 15 mins., 92.96% after 48 hrs. and 95.41% after 96 hours. The mean and standard deviation shows highly significant value in all the time period ($p < 0.001$). It may be attributed to the reasons that both suffice to the potentiality of both irrigants. EDTA and Chlorhexidine act synergistically.²⁸ Though there was not much research regarding the use of chlorhexidine and EDTA combination as irrigating solution. From the findings of this study, we can evaluate the use of these as irrigating solution. From the findings of this study, we can evaluate the use of these as irrigating solution.

In the control group though there was reduction of bacteria in pre and post irrigation group, but there was not much difference found after 48 and 96 hours. So the reduction percentage for this group was found 60.84% after 15 mins., 65.62% after 48 hrs. and 57.55% after 96 hrs. The mean and standard deviation in different time group and statistical analysis was found to be not significant (Table VII). The decrease of root canal flora in pre and post irrigation may be due to its ability to flush out debris from the root canal rather than having any anti-microbial property. It is also noted that after repeated irrigation with saline, microorganism culture remained positive.

Within the limitations of this study, it may be concluded that the most suitable irrigating solution would be the combination of 0.2% chlorhexidine and neutral EDTA followed by 0.2% Chlorhexidine, then 0.9% normal saline and lastly neutral EDTA. Since this study was conducted in small sample size with few irrigating solutions further study may be needed with larger sample with different irrigating solution in varied time period and concentration to come up with definite conclusion.

REFERENCES

1. Abraham S, Raj JD, Venugopal M. Endodontic irrigants: a comprehensive review. *J Pharm Sci & Res* 2015;7(1):5-9.
2. Abirami CP, Rao SCV. Evaluation of antibacterial activities of root canal irrigants: an in-vitro study. *Journal of Endodontology* 1999;11:27-29.
3. Torabinejad M, Handysides R, Khademi AA, et al. Clinical implications of the smear layer in endodontics: a review. *Oral Surg Oral Med Oral Pathol Radio Endod* 2002;94(6):658-66.
4. Zamany A, Safavi K, Spangberg LS. The effect of chlorhexidine as an endodontic disinfectant. *Oral Surg Oral Med Oral Pathol Radio Endod* 2003;96(5): 578-81.
5. Hill PK. Endodontics. *Journal Prost Dent* 1959;9:142.
6. Ingle JI, Zeldow BJ. An evaluation of mechanical instrumentation and irrigative culture in endodontic therapy. *JADA* 1958;57(4):471-6.

7. Aurenbach NB. Antibiotic versus instrumentation in Endodontics. *N Y State Dent Journal* 1953;19:225-8.
8. Griffiee MB, Patterson SS, Miller CH, et al. Relationship of bacteriodesmelaninogenic to symptoms associated with pulpal necrosis. *Oral Surgery Oral Medical Oral Pathology* 1980;50(5):457-61.
9. Gutierrez Jh, Villena F, Jofre A, et al. Bacterial infiltration of dentin as influenced by proprietary chelating agents. *J Endod* 1982;8(10):448-54.
10. Cruickshank R, Duguid JP, Swain RHA. Medical microbiology. A guide to the laboratory diagnosis and control of infections. Edinburgh, London, U.K: E & S Livingstone Ltd 1965.
11. Darrag AM. Effectiveness of different final irrigating solution on smear layer removal in intraradicular dentin. *Tanta Dental Journal* 2014;11(2):93-99.
12. Grossman Li. Root canal therapy. 4th edn. Philadelphia: Lea and Febiger 1955:202.
13. Hashem AAR, Ghoneim AG, Lutfy RA, et al. The effect of different irrigating solutions on bond strength of two root canal-filling systems. *J Endod* 2009;35(4):537-40.
14. Balaji TS. Effect of various root canal irrigants on removal of smear layer and debridement: an SEM study. *Pak Oral Dental J* 2010;30:205-11.
15. Yelsilsoy C, Whitaker E, Cleveland D, et al. Antibacterial and toxic effects of established potential root canal irrigants. *Journal of Endodontics* 1995;21(10):513-5.
16. Engstrom B, Lunberg M. The correlation between positive culture and the prognosis of root canal therapy after pulpectomy. *Odontol Rev* 1965;16(3):193-203.
17. Sundqvist G. Bacteriological studies of necrotic dental pulp. Dr. Odont thesis, University of Umea, Umea, Sweden 1976.
18. Davies A. The mode of action of chlorhexidine. *Journal of Periodontology Res* 1973;8(S12):68-75.
19. Nicholls SE. The efficacy of cleansing of root canal. *British Dent J* 1962;112:167-70.
20. Fardai O, Turnbull RS. A review of literature on use of chlorhexidien in dentistry. *JADA* 1986;112(6):863-9.
21. Zamany A, Spangberg LS. An effective method of inactivating chlorhexidine. *Oral Surg Oral Med Oral Path Oral Radio Endod* 2002;93(5):617-20.
22. Patterson S. In vivo and in vitro studies on the effect of the disodium salt of ethylene diamine tetra-acetate on human dentin and its endodontic implication. *Oral Surgery Med Oral Path* 1963;16:83-103.
23. Vahdaty A, Ford PTR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. *Endodontic Dental Traumatol* 1993;9(6):243-8
24. White R, Goldman M. Influence of the smeared layer on dentinal tubule penetration by various root canal sealers. *Jour Dent Res* 1983;62(417):215.
25. Zehnder M, Schmidlin P, Sener B, et al. Chelation in root canal therapy reconsidered. *J Endod* 2005;31(11):817-20.
26. Nikiforuk G, Sreebny L. Demineralization of hard tissue by organic chelating agents at neutral pH. *Journal Dental Restoration* 1953;32(6):859-67.

27. Heling B, Shapirdo S, Sciaky I. An invitro comparison of the amount of calcium removed by the disodium salt EDTA and hydrochloric acid during endodontic procedures. *Oral Surgery Oral Medical Oral Path* 1965;19(4);531-3.
28. Dalton GW. Chlorhexidine. In: Block SS. *Disinfection, sterilization and preservation*. 4th edn. Philadelphia: Lea and Febiger 1991:274-89.