COMPARATIVE EVALUATION OF CLEANING EFFICACY IN DIFFERENT IRRIGATION FLUIDS AND TAPER OF ROTARY SYSTEM: IN VITRO STUDY

Amir Arsalan Navabi DDS. MS[1], Paridokht Zarean DDS[2], Parichehr Zarean DDS[3*]

[1] Assistant professor, Department of Endodontics, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

[2,3] Dentist, Member of Young Researchers and Elite Club, Isfahan (khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

Abstract

Introduction: Bacteria and their byproducts are etiologic factors that cause endodontic treatment failure. Reduction of root canal bacterial contamination is one of the main aims of root canal therapies. The purpose of this study is to compare the effectsof different irrigation fluids and rotary file tapers on root canal cleanliness.

Material and Method: Root canals of 72 human upper lateral incisors were enlarged to ISO #20 K-file, inoculated with Enterococcus faecalis for 72 hours and divided into six experimental groups and prepared with Flexmaster 0.02, 0.04, 0.06 tapers size 30. Control groups (n=10) were divided in two groups with and without bacterial inoculation and no mechanical instrumentation. Cleaning efficacy was evaluated in terms of reduction of colony forming units. T-test, ANOVA and Tuckey test was done between groups.

Results: Canals instrumented with 0.06 taper have greater reduction in CFUs than canals instrumented with 0.04 and 0.02 taper ($p<0.001$). 0.04 taper has also greater reduction in CFUs than 0.02 taper ($p<0.001$). No significant differences were also observed between two irrigation fluids.

Conclusion: Under the condition of this study, root canal preparation with greater taper affected canal cleanliness and better debridement.

Key words: Enterococcus faecalis, Flexmaster rotary file, Tapering, Cleaning efficacy.

Introduction

Bacteria and their byproducts have a principle role in pulpal and periapical pathogenesis (1,2); once infected root canal space harbors a plethora of bacteria, growing mostly in sessile biofilms, aggregates, coaggregates and planktonic cells.(3,4,5) It seems reasonable that elimination or reduction of pathogenes as a prime objective in successful treatment of apical periodontitis leads to successful treatment of this condition. In fact, when previously infected canals are rendered negative to bacterial sampling, an improved prognosis has been suggested.(6,7)

Among bacteria, Enterococcus faecalis is the most consistently reported organism from former cases. This organism is resistant to most of intracanal medicaments and can tolerate up to a pH of 11.5.(8) E. Faecalis can also survive prolonged starvation and grow as a monoinfection in treated canals in defiance of the absence of synergistic support from other bacteria.(9) Moreover, Endodontic infections with E. faecalis are usually difficult to eliminate even with intracanal calcium hydroxide dressing (10).

Reduction in bacterial count is accomplished by a triad of mechanical instrumentation such as cleaning and shaping with various irrigating solutions, and disinfection with intracanal medicaments.(4,11,12) In the time that endodontic instruments were manufactured by ISO, it was believed that the only way to reach the irrigation fluids to the critical apical 3 mm of the root canal, was apical preparation as wide as possible in order to reduce the microbial population and cleanliness.(13,14,15,16,17) Nowadays, by manufacturing of nickel-titanium rotary systems, it is suggested that increasing root canal taper should be done with apical preparation as narrow as possible.

Buchanan refers to advantages of Variable Taper as a tapering with easy and simple use, enhanced and predictable cleaning and obturation outcomes even in inexperienced hands, adequate coronal enlargement, full deep shape, and apical resistance form in a simple instrument sequence (18). In his recent study, de Gregorio et al assessed only the effect of apical size and taper on irrigate volume delivery without analyzing the effect of irrigate solution in cleanliness (19).

Having a rather similar study to ours, Ioanna et al, akhlaghi et al, Cohenca et al, Moshari et al. investigated the effects of taper on root canal cleanliness. Our detailed methodology, however, is different from theirs. (20,21, 2, 22, 23)

The purpose of the present study, therefore, is to investigate the effects of different irrigation fluids and taper of rotary system on root canal cleanliness, which is evaluated in terms of reduction of colony forming units (CFUs) in 3 mm apical of the powdered root. The null hypothesis is that the increase in taper does not affect root canal cleanliness.

Material and method

Eighty-two human incisors with one root canal, extracted due to orthodontic or periodontal reasons, were collected. Before preparation, all teeth were radiographed in buccolingual and mesiodistal direction to rule out teeth with any aberrant canal morphology, and to confirm a single canal. Teeth with severe root curve, crack or fractured root, calcified root, immature apex, decayed and filled teeth were excluded.

Preparation of roots

The teeth were cleaned right after the extraction and debridement. Then, they were placed in sodium hypochlorite 5/25% for 30 minutes in order to surface disinfect and were stored in sterile normal saline 0/9% (daroopakhsh, Tehran, iran). In the first place, the teeth were cut from CEJ perpendicularly to their long axis by using a diamond disk (Horico H557F220) 12 mm from the root tip. Patency of the roots and single canals were ensured by using #10, #15 K-file (Densply/Maillefer, Ballaigues, Switzerland). To reduce confounding variables, all samples were primarily instrumented to #20 K-file (Densply/Maillefer, Ballaigues, Switzerland), under copious irrigation with distilled water into 11 mm working length. Then, roots were soaked in EDTA 17% (Vericom, Korea) for 10 minutes followed by sodium hypochlorite 5.25% (for 10 min) and finally rinsed with sterile water. After preparation, the roots were randomly divided into six experimental groups (n=12) and two control groups (n=5).

Sterilization of samples

All samples were placed in brain-heart infusion[1] broth and autoclaved for 20 minutes in 121 °C and 15 psi. All samples were autoclaved again in the same way as the first time for better accuracy. To assure sterilization, all samples were

[1] BHI

incubated separately in micro tube contain BHI for 24 hours in aerobic and aseptic condition at 37 °C. If micro tube became dark, it would mean sterilization is incomplete and the process of sterilization must be repeated.

Preparation of Enterococcus faecalis

In this study, in order to create standard and controlled infection in all cases, we used resistant bacteria and cause of endodontic treatment failure called enterococcus faecalis. Therefore, this Gram-positive anaerobic bacteria was prepared from the Department of Microbiology, Faculty of Tehran medical University with ID number ATCC-29212.

Contamination of samples

A suspension of bacteria was prepared by adding 1 mL pure culture of Enterococcus faecalis (ATCC2912) grown in brain heart infusion broth. 0.05 cc of suspension was injected in each canal of experimental group and positive control group. The access cavity was sealed with intermediate restorative material Cavit (Premier Dental Products Co., Philadelphia, PA). The roots were incubated at 37°C for 72 hours separately.

Preparation of root canals

The samples were kept sterile and divided into six groups (n=12) after the incubation period. Preparation steps were performed as follows:

In all the groups, recapitulation with #15 K-file (Densply/Maillefer, Ballaigues, Switzerland) between different instruments was kept standard and after each file 2 ml of irrigate was flushed. Canal preparation was done by single length technique with Flexmaster rotary instruments.

Group I. prepared with this sequence: Flexmaster 0.02 taper size 30, Flexmaster 0.04 taper size 30, Flexmaster 0.06 taper size 30 with 2ml NaOCl 2.5% irrigation after each file

Group II. prepared with this sequence: Flexmaster 0.02 taper size 30, Flexmaster 0.04 taper size 30 with 2ml NaOCl 2.5% irrigation after each file.

Group III. prepared with this sequence: Flexmaster 0.02 taper size 30 with 2ml NaOCl 2.5% irrigation after each file.

Group IV. prepared with this sequence: Flexmaster 0.02 taper size 30, Flexmaster 0.04 taper size 30, Flexmaster 0.06 taper size 30 with 2ml Normal saline irrigation after each file.

Group V. prepared with this sequence: Flexmaster 0.02 taper size 30, Flexmaster 0.04 taper size 30 with 2ml Normal saline irrigation after each file.

Group VI. prepared with this sequence: Flexmaster 0.02 taper size 30 with 2ml Normal saline irrigation after each file.

Group VIIa. No instrumentation was performed after bacterial inoculation.

Group VIIb. Neither bacterial inoculation nor mechanical instrumentation was performed.

Preparation for microbiologic evaluation

After canal preparation and final rinsing with 10ml distilled water, 3mm apical of root was powdered with carbide bur and transferred into tubes containing sterile BHI. After 10-fold serial dilutions in saline solution, aliquots of 0.1 mL were plated onto nutrient agar plates and incubated at 37°C for 48 hours. The colony-forming units (CFUs) were counted after 24 hours of growth.

Results

The comparison between groups, according to two way-ANOVA test with regard to reaction, showed no interplay between NaOCl 2.5% and Normal saline groups. However, one way-ANOVA test showed significant differences between groups.

To assimilate the quantity of E.faecalis in prepared tooth with 0.02, 0.04, 0.06 taper and NaOCl 2.5% as irrigate, Duncan test was used. The test showed significant differences and suggests that high tapering reduce bacteria more than lowers. Different taper in Normal saline groups had the same result as NaOCl group.

The quantity of E.Faecalis in prepared teeth with 0.02 taper, NaOCl and Normal saline as irrigation was assessed by T-test and showed no significant differences (Pvalue=0.27). 0.04 and 0.06 taper with different irrigation also showed no significant difference with Pvalue 0.42 and 0.33 respectively.

Canals instrumented with 0.06 taper have greater reduction in CFUs than canals instrumented with 0.04 and 0.02 taper ($p<0.001$). Instrumentation with 0.04 taper also

has greater reduction in CFUs than canals instrumented with 0.02 taper ($p<0.001$). Thus, canals instrumented with greater taper were cleaner and there is no significant difference between two irrigation fluids. The number of CFUs in each of the specimens in different groups is shown in Table 1.

Discussion

The goal of root canal therapy is to remove all necrotic and vital organic tissue and neutralize any potential antigenic materials remaining in the canal space by mechanical instrumentation and irrigation from the root canal system. It also prepares the canal system for an easy placement of medicaments and obturation.(24,25,26) The objective of the present study is to evaluate the influence of different irrigation fluids and taper of rotary system on root canal cleanliness. To achieve this, NaOCl and Normal saline were used as irrigation fluids separately. The canals studied had the same apical preparation size but with different tapers.

Sundquist et al reported that 38% of failed root canal teeth contaminated with E.faecalis.(26) As this organism is heat resistant, it can survive in temperatures of even 60°C for 30 minutes and thrive alone successfully (27).

In the present study, thus we used Bile Esculin agar since it allows the growth of enterococci and streptococci, including E. faecalis.

Irrigation fluids

Since it has no antibacterial effects on E. faecalis, Sterile distilled water was used as one of the irrigates in this study.; and NaOCl 2.5% can display its effect as irrigate. Based on the findings of our study on irrigation, NaOCl 2.5% and Normal saline did not show significant differences.

Elimination of bacteria perhaps could be solely attributed to mechanical action of instruments. It is in accordance with Shabahang et al that suggest NaOCL 2.5% had no bactericidal effect on E. faecalis.(28) It is thus, suggested that NaOCl 2.5% has the same role as Normal saline which flushes action. Moreover, it results in egression of debris that are contaminated with canal bacteria.

Different taper of rotary system

Another finding of our study about different taper is that root canal taper affected its cleanliness. This result can be compared with some previous studies, done by considering both size and taper. Akhlaghi et al. evaluated #25, #30, #35 MAF sizes and 0.04, 0.06 taper of RaCe rotary files on root canal cleanliness and suggested that the greater size and/or taper resulted in reduction of bacteria and acceptable debridement, although differences between the groups were not significant.(2) On the other hand, Albrecht et al. reported that canals prepared with GT file #20, taper increasing led to better debridement. But when the MAF size was #40, no effect of taper was observed.(29) In this regard, Usman et al. sought to investigate the effect of flaring in bacterial elimination. They extracted teeth after cleaning and shaping by Use of #20 and #40 GT files 0.06 taper and then cut them perpendicularly 0.5, 1.5 and 2.5 mm from the apex. It was shown that apical preparation up to #20 left more debris than #40. Eventually, it was found that the greater apical size will lead to more cleanliness. The priority of their study among other similar studies, is that their research was accomplished on human teeth (in vivo) and amount of debris were examined by histologic evaluation, therefore it can be more accurate.(30)

The result of Lumley et al. suggests that prepared canals up to #60 K-file by step-back method were considerably cleaner than canals prepared up to #35 K-file. By use of high taper files, we have also more appropriate shaping in addition to better canal cleanliness.(31)

To achive the aim of this study, we evaluate studies that prepare canals with same size and different tapers. Van der Sluis et al. and Lee et al. prepared root canals with GT files #20, and suggest that taper increasing led to better debridement. (32,33)

Singla et al. reported substantial decrease in bacterial count with progressively larger tapers. (34) Lumely et al. also reported that canals shaping with hand files of greater taper were significantly cleaner. (35) These finding are in agreement with the findings of Dalton et al., Siqueira et al., Byström and Sundqvist. (10,29,35)

According to Buchanan study, more coronal tapering leads to better flushing of irrigants to the apical region. They suggest that high tapered canals leads to better

obturation and then arrested the remained bacterias. (17) The viewpoint about the remained bacteria was supported by Peters. (36)

Boutsioulcis et al. assessed the effect of canal tapering on irrigation flushing by use of Computational Fluid Dynamic model. According to their results, increase in canal tapering led to increase in the flow of irrigation fluid and Shear stress to the walls of canal. While it decrease the risk of irrigation fluid extrusion, which can cause greater impact to remove the bacteria. (37)

The results of Siqueria et al. were opposed to the results of our study. They suggest that canal preparation with different Tapering (0.02, 0.06 and 0.012) had no effect on further reducing amount of bacteria in root canals. (38) The use of mandibular premolars in this research is one of the reasons that may cause no significant difference in the results of their study. The cross-section of mandibular premolars are oval shape and are wider in buccolingual direction. Despite of the two instrumentation techniques that were performed in this study, the oval walls may remain without filling. In order to solve this problem, anterior teeth with single root canals and circular cross-section anatomy were used in our study.

Ioanna et al. also suggested root canals prepared with GT files #30 and taper 0.04, 0.06, 0.08, showed no significant differences between groups of different taper and concluded that taper can affect root canal cleanliness only when final instrument size is smaller than 30; But stated this claim requires further investigation. (19)

The results of our study are in consistent with Singla's study. (39) Results showed more Tapering (Protaper F4 and 0.06 taper) removed more bacteria from the canal than less tapered. In this study, paper point was used for the sampling of canal; but it can only absorb the liquid contents in the canal and may not absorb bacteria that were found in some parts of root canal walls.

There are several reported methods of endodontic instrumentation technique evaluation, including microscopic observation of remaining debris, morphometric analysis and a bacteriological assessment with samples which were taken with paper points or piezo drills. (40, 41, 42)

The result of our study was in agreement with Lumley's. (35) They evaluate the cleanliness of canal that flared with different sizes of files manually by an optical microscope observation.

None of the previous studies considered the penetration of bacteria into dentinal tubules. Hence, we were encouraged to plan a slightly similar study with a different and accurate methodology. Our study was therefore, done by powdering 3 mm root end with carbide bur. In this way, all of the bacteria in the canal walls and dentinal tubules were transported to the Medium. This method is more effective and the results are more accurate.

It is noteworthy that the strength of teeth with root canal treatment directly depends on the remaining intact tooth structure. Endodontic treatment processes, such as access cavity preparation and instrumentation cause to loss of tooth structure and weak root canal walls.

Rundquist study showed that increasing the taper of the canal, reduced stress during the abturation. But after that, occlusal forces increasing the stress in cervical part of teeth and then vertical root fracture rise from this part. (43)

The finding of our research showed high tapering is more effective in root canal cleanliness, which would reduce the bacterial count to close to 0. However, other clinical effects of larger instrumentation, including compromised restorability, fracture susceptibility, and canal path alterations must also be considered when using any instrumentation technique.(44) Thus, root canals should be flared as needed and prepared in a conical shape with a gentle taper.

It is suggested to plan a study which evaluates the effect of different tapers on VRF strength of the root and canal path alterations.

References

[1] Kakehashi S,Stanley HR,Fitzgerald RJ.The Effect of surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats.Oral Surg Oral Med Oral Pathol.1965;20:340-9.

[2] Mohammadzadeh Akhlaghi N, Rahimifard N, Moshari A, Vatanpour M, Darmiani S. The Effect of Size and Taper of Apical Preparation in Reducing Intra-Canal Bacteria: A Quantitative SEM Study. Iran Endod J. 2014 Winter;9(1):61-5.

[3] Baumgartner JC, Falkler WA. Bacteria in the apical 5 mm of infected root canals. J Endod. 1991;17:380-3.

[4] Block RM, Bushell A, Rodrigues H, Langeland K. A histopathologic, histobacteriologic, and radiographic study of periapical endodontic surgical specimens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1976;42:656-78.

[5] Barbizam JVB, Fariniuk LF, Marchesan MA, Pecora JD, Sousa- Neto MD. Effectiveness of manual and rotary instrumentation techniques for cleaning flattened root canals. J Endod. 2002; 28:365-6.

[6] EngstrOm B, H&rd AF, Segerstad L, Ramstr6m G, Frostell G. Correlation of positive cultures with the prognosis for root canal treatment. Odontol Revy. 1964;15:257-70.

[7] Oliet S, Sorin SM. Evaluation of clinical results based upon culturing root canals. J Br Endod Soc. 1969;3:3-6.

[8] Bystrom A, Claeson R, Sundqvist G: The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals phenol. Endod Dent Traumatol. 1985 Oct;1(5):170-5.

[9] FabriciusL , Dahl€n G, Holm SC,M oller AIR: Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res. 1982 Jun;90(3):200-6.

[10] Güven K, Orstavik D. Virulence factors of enterococcus faecalis: relationship to endodontic disease. Crit Rev Oral Biol Med. 2004;15:308-20.

[11] Siqueira JF Jr, Rôças IN, Paiva SS, Guimarães-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104:122-30.

[12] Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res. 2002;81:761-6.

[13] Nair P, Sjogren U, Krey G, Kahnberg K, Sundqvist G. Intraradicular bacteria and fungi in root-filled asymptomatic human teeth with therapy resistant periapical lesions: a long-term light and electron microscopic follow up study. J Endod. 1990;16:580–8.

[14] Orstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J. 1991;24:1–7.

[15] Simon JH. The apex: how critical is it. Gen Dent. 1994;42:330–4.

[16] Wu MK, Wesselink PR. Efficacy of three techniques in cleaning the apical part of curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;79: 492–6.

[17] Siqueira J, Araujo M, Garcia P, Fraga R, Saboia Dantas C. Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of the root canals. J Endod. 1997;23:499–502.

[18] Buchanan LS. The standardized-taper root canal preparation: part 1-concepts for variably tapered shaping instruments. Int Endod J. 2000;33:516–29.

[19] de Gregorio C, Arias A, Navarrete N, Del Rio V, Oltra E, Cohenca N. Effect of apical size and taper on volume of irrigant delivered at working length with apical negative pressure at different root curvatures. J Endod. 2013 Jan;39(1):119-24.

[20] Ioanna S. Arvaniti, DDS. MSc, Marouan G. Khabbaz, DDS. PhD. Influence of Root Canal Taper on Its Cleanliness: A Scanning Electron Microscopic Study. Journal of Endodontics. June 2011;37(6):871–874.

[21] Akhlaghi NM, Dadresanfar B, Darmiani S, Moshari A. Effect of master apical file size and taper on irrigation and cleaning of the apical third of curved canals. J Dent (Tehran). 2014 Mar;11(2):188-95.

[22] Cohenca N, Paranjpe A, Heilborn C, Johnson JD. Antimicrobial efficacy of two irrigation techniques in tapered and non-tapered canal preparations. A randomized controlled clinical trial. Quintessence Int. 2013 Mar;44(3):217-28.

[23] Moshari AA, Akhlaghi NM, Rahimifard N, Darmiani S. Reduction of Enterococcus faecalis in curved root canals after various sizes and tapers of canal preparation. J Conserv Dent. 2015;18:306-9

[24] Negishi J, Kawanami M, Ogami E. Risk analysis of failure of root canal treatment for teeth with inaccessible apical constriction. J Dent. 2005;33:399-404.

[25] Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004;15:348-81.

[26] Sundqvist G,Figdor D,person S,Sjogren U.Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment.Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85(1):86-930.

[27] Byström A, Sundqvist G. Bacteriological evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981;89:321-8.

[28] Shabahang S,Torabinejad M: Effect of MTAD on Entrococcus faecalis-contaminated root canals of extracted human teeth. J Endod. 2003;29:576-579.

[29] Albrecht LJ, Baumgartner JC, Marshal JG. Evaluation of apical debris removal using various sizes and tapers of ProFile GT files. J Endod. 2004;30:425–8.

[30] Usman N, Baumgartner JC,Marshall JG.influence of instrument size on root canal debridement.J Endod. 2004; 30(2):110-2.

[31] Lumley PJ.Cleaning efficacy of two apical preparation regimens following shaping with hand files of greater taper.Int Endod J. 2000;33(3):262-5.

[32] Lee SJ, Wu MK, Wesselink PR. The efficacy of ultrasonic irrigation to remove artificially placed debris from different-sized simulated plastic root canals. Int Endod J. 2004;37:607–12.

[33] Van der Sluis LWM, Wu MK, Wesselink PR. The efficacy of ultrasonic irrigation to remove artificially placed dentine debris from human root canals prepared using instruments of varying taper. Int Endod J. 2005;38:764–8.

[34] Singla M,Aggarwal V, Logani A, Shah N. Comparative evaluation of rotary Protaper,Profile and conventional step back technique on reduction in Enterococcus faecalis colony-forming units and vertical root fracture resistance of root canal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109:105-110.

[35] Dalton CB, Orstavik D, Phillips C. Bacterial reduction with nickel-titanium rotary instrumentation. J Endod. 1998;24:763-7.

[36] Peters LB,Wesselink PR,Moorer WR.The fate and the role of bacteria left in root dentinal tubules.Int Endod J. 1995; 28(2):95-9.

[37] Boutsioukis C, Gogos C, Verhaagen B, Versluis M, Kastrinakis E, van der Sluis LWM. The effect of root canal taper on the irrigant flow: evaluation using an unsteady Computational Fluid Dynamics model. International Endodontic Journal. 2010;43:909–916.

[38] Siqueira JF , Jr., Lima KC, Magalhaes FA, Lopes HP ,de Uzeda M. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod. 1999;25(5):332-5.

[39] Mamta Singla,Vivek Aggarwal, Ajay Logani, Naseem Shah.Comparative evaluation of rotary Protaper,Profile and conventional step back technique on reduction in Enterococcus faecalis colony-forming units and vertical root fracture resistance of root canal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:105-110.

[40] Tabrizizadeh M, Shareghi A. The Effect of Preparation Size on Efficacy of Smear Layer Removal; A Scanning Electron Microscopic Study. Iran Endod J. 2015 Summer;10(3):169–173.

[41] Singh M, O. Kasat V. Efficacy of various combinations of irrigants and medicaments on Candida albican: An in vitro study. J Int Soc Prev Community Dent. 2015 May-Jun;5(3):157–162.

[42] Paranjpe A, de Gregorio C,Gonzalez A, Cohenca N. Efficacy of the Self-Adjusting File System on Cleaning and Shaping Oval Canals: A Microbiological and Microscopic Evaluation. Journal of endodontics. Feb2012;38(2):226-31.

[43] Rundquist BD,Versluis A. How dose canal taper affect root stresses. Int Endod J. 2006;39(3):226-37.

[44] Guppy D, Curtis R, Ford T. Dentin chips produced by nickeltitanium rotary instruments. Endod Dent Traumatol. 2004;16:258-64.