Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies

NLM ID: 101716117

Index Copernicus value = 91.86

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI)

(e) ISSN Online: 2321-9599;

(p) ISSN Print: 2348-6805

Original Research

Comparative evaluation of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: An in vitro study

¹Pankaj Kumar, ²Gurleen Kaur

¹Associate Specialist, Dental Department, Tata Main Hospital, Jamshedpur, Jharkhand, India; ²Endodontist and Private Practitioner, Bihar, India

ABSTRACT:

Background: E. faecalis is one of most common microorganisms present within the root canals. It is commonly cultured from root canals that have exhibited reinfection or improper instrumentation. Use of proper irrigating solutions along with technique used is a major prerequisite for infection control in endodontic infections. Aim: The study aimed to compare the three irrigation techniques (i.e., conventional needle, EndoVac system and rotary instrumentation). Materials and methods: This experimental in vitro analysis was conducted on sixty single rooted extracted teeth that comprised of a test group. Access opening was done in all the teeth and root canals were then enlarged till file size # 20. All prepared teeth were inoculums of E. faecalis for up to seventy two hours. These teeth were then categorized in 3 groups i) Group I: In this test group, enlarged root canals conventional needle and syringe was used for irrigation purpose; ii) Group II: Root canals within this particular group underwent irrigation by means of EndoVac system needles and iii) Group III: In this particular group, root canals had been irrigated after instrumentation using Flexmaster rotary endodontic system. 5 % Sodium hypochlorite (NaOCl) solution was commonly used as an irrigating solution in all the study groups. Commercial available (ATCC-29212) strain of E. faecalis was acquired for inoculation of root canals of all the endodontically prepared teeth. Culture media used for E. faecalis was 'Bile esculin' agar media. Disinfection efficacy of all techniques was evaluated by observing colony forming units (C.F.Us) using manual method. Mean ± standard deviation values were derived and study groups were then compared by means of 'two-way' ANOVA test. Significance level was kept at P value of 0.05. Results: On analyzing the obtained mean ± SD values of E. facalis colony forming units, Group I studied root canals that were irrigated by making use of conventional needle and syringe demonstrated 114.76 ± 20.19 C.F.U.s whereas, Group II root canals that were irrigated by making use of Endo Vac system of irrigation needle demonstrated mean 99.12 Colony Forming Units ± 13.22 (standard deviation). While on the other hand, in root canals that were irrigated with 'Flexmaster' Rotary endodontic system, mean \pm SD score of Colony forming unit count of 56.54 ± 11.81 were observed. On intra-group comparison using Two-Way ANOVA statistical tool, Groups I and II had P value = 0.05 (statistically significant); Groups II and III on intra-group statistical comparison showed statistically significant P value of 0.04 whereas, a P value of 0.01 (statistical significance) was obtained on comparing I with Group III. Conclusion: On evaluating the three irrigating techniques i.e., conventional use of needle and syringe that uses positive pressure; EndoVac system that utilizes negative apically directed pressure due to presence of lateral vent and use of irrigation while using rotary endodontic file system, it was notable that use of rotary instrumentation aided in reduction of E. faecalis colony count most efficiently.

Keywords: Conventional, EndoVac, rotary, irrigation, technique, sodium hypochlorite, E. faecalis, C.F.U.s

Received: 22 June, 2022

Accepted: 25 July, 2022

Corresponding author: Pankaj Kumar, Associate Specialist, Dental Department, Tata Main Hospital, Jamshedpur, Jharkhand, India

This article may be cited as: Kumar P, Kaur G. Comparative evaluation of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: An in vitro study. J Adv Med Dent Scie Res 2022;10(8):110-114.

INTRODUCTION

Bacterial metabolic products play a significant function role in the pathogenesis of pulpal and periapical diseases.^[1-5] Eliminating and/or decrease in pathogenic bacteria remain to be the primary aim in successfully treating apical periodontitis. An improvement in prognosis is achieved when the infected root canals demonstrate negative culture on culturing samples. ^[6,7]

Among all bacterial species found within root canal systems, *Enterococcus faecalis* has been consistently reported. This microrganism has shown resistance towards most of the intra-canal medicaments. This particular bacteria is capable of surviving till 11.5 pH. ^[8] *E. faecalis* survives long periods of starvation. It

can show growth as mono-infection within already treated root canals with no synergism with other bacterial species. ^[9] Root canal infections due to *E. faecalis* are difficultly eliminated even following intra-canal dressing using calcium hydroxide.^[10]

E. faecalis is reported as most common bacterial species that has been isolated from root canals with endodontic infection following treatment. This particular microorganism has an ability for survival within a closed environment where there is scant availability of nutrients along with less existence of commensal inter-relationship with variety of root canal bacteria. E. faecalis is capable of forming microbial biofilms over the walls of root canals. This organism can infiltrate dentin tubules for an approximate depth measuring up to 653 µm. Hence, it is quite possible that the bacterial colonies existing between and within the dentin tubules are capable of surviving chemo-biomechanical root canal instrumentation along with medications or agents used for irrigating these canals. [11]

The ultimate aim of endodontic therapy is elimination of various microbial organisms from within root canal systems as well as from radicular portion of dentin. Infact, the most frequent reason behind the failure of root canal or endodontic treatment is most often inadequate and less effective elimination of root canal bacterial cells. This is most commonly due to the complicated root canal morphology and aggregation of intra root canal bacterial population within the canal biofilm coverings.

A decrease in bacterial cell counts can be achieved by combination of bio-mechanical canal preparation, use of irrigating agents and disinfection using intracanal medications.^[12,13,14]

Sodium hypochlorite is commonly used root canal irrigating agent owing to its anti-microbial properties and capability of tissue dissolution. ^[15] Although there are certain drawbacks towards sodium hypochlorite use such as unpleasant taste and potential for irritation of peri-apical tissues.

In conventionally used irrigation technique using needle, both replenishment as well as exchange of irrigating solution in apical 3rd as well as effective chemical cleaning is dependent upon the depth of penetration of irrigating needle. Irrigant exchange usually does not flow beyond the tip of an irrigating needle i.e., the fluid flow does not take place beyond 1 to 1.5 mm beyond any irrigating needle with vents on its sides. ^[16,17] Hence, conventional method of irrigation using needle that works on positive pressure can only effectively clean root canal(s) in coronal part however, it is not effective in apical portion of root canal. ^[18]

EndoVac, an irrigation system (Discus Dental, Smart Endodontics, Culver City, CA) that uses negative pressure which is directed apically comprises of three parts: a) Micro cannula; b) macro cannula and c) master delivery tip (or MDT). The microdelivery tip works by simultaneous delivering and evacuation of irrigating solution. ^[19]

The macro-cannula suctions the irrigating solution from chamber to coronal as well as middle portion of root canals. The micro-cannula contains twelve microscopic holes which are capable in evacuation of root canal debris till complete working length. ^[20]

It was earlier believed that for the irrigating fluids to reach up to the critical length at 3 mm at the apex of a root canal, the canal preparation at apical end should be as wide enough for reducing population of microbes and for making the canal sterile. However, after advent of nickel-titanium endodontic rotary systems, it has been suggested that apical preparation of root canals must be narrow. ^[21]

Hence, this study was undertaken to evaluate three irrigation techniques: Positive apical pressure based conventional use of needles, negative apical pressure based EndoVac system and irrigation during instrumentation using Flexmaster rotary file system.

MATERIALS AND METHODS

This was an experimental and in vitro study conducted using 60 single rooted extracted teeth as test group. All root canals were enlarged till size 20 files. All teeth were then sterilized using autoclaving and were then inoculated with E. faecalis for a total duration of 72 hours. All teeth were categorized into three groups a) Group I: In this group, root canals were irrigated using conventional needle; b) Group II: Root canals in this group were irrigated using EndoVac needles and c) Group III: In this group, root canals were irrigated following instrumentation with Flexmaster rotary endodontic system. 5 % Sodium hypochlorite (NaOCI) solution was used as irrigating agent in all three groups.

Commercial strains of E. faecalis, ATCC-29212 was obtained for inoculating the root canals of prepared teeth. 'Bile esculin' agar media was used for culturing of *E. faecalis*. Bacterial suspension was then prepared by addition of 1 millilitres of pure *E. faecalis* culture (ATCC2912) which was initially cultured in Brain Heart Infusion (BH) broth; Equal volumes (0.05 mL) suspension was injected within prepared root canals using a volume sampler. Following this, all access cavities was coronally sealed using an intermediate restorative material- Cavit (Premier Dental Products Co, Philadelphia, USA). All prepared tooth sample were then incubated at a temperature of 37°C for total duration of 72 hours.

Disinfection efficacy of each studied root canal was evaluated by assessing colony forming units or C.F.Us by manual method for counting of colonies. Mean \pm standard deviation (SD) values were obtained and groups were compared using two-way ANOVA test. Significance level was set at 0.05.

RESULTS AND OBSERVATIONS

On analyzing the mean \pm SD values of colony forming units (CFUs), Group I root canals that were

irrigated using conventional needles showed 114.76 \pm 20.19 C.F.U.s while Group II wherein root canals were irrigated using EndoVac needle with 99.12 \pm 13.22 Colony Forming Units. On the other hand, root canals which were irrigated along with Flexmaster

Rotary system demonstrated mean \pm SD values of Colony forming units of 56.54 \pm 11.81 (table 1 and Graph 1). P values of 0.05; 0.04 and 0.01 were obtained on comparison between Groups I and II, II and III and I and III (table 2).

Table 1: Table showing mean colony forming units in three study groups and P value

Group I (conventional technique)	Group II (Endo Vac)	Group III (Flexmaster Rotary endodontic system)
114.76 ± 20.19	99.12 ± 13.22	56.54 ± 11.81

Table 2: Table showing P values

Between Groups I and II	Between Groups II and III	Between Groups I and III		
0.05 (significant)	0.04 (significant)	0.001 (highly significant)		

Graph 1: Graph showing mean colony forming units or C.F.U.s in studied groups



Hence, the best results for disinfection of root canals were obtained using FlexMaster rotary system, which was followed by EndoVac needle system. Least disinfection was seen with conventional method of use of needle.

DISCUSSION

Elimination of intra-canal microbial population either completely or partially while at the same time making use of irrigating materials that exhibit compatibility with peri-radicular tissues and aid in their healing is the biggest microbiological aim of chemo-mechanical root canal preparation. ^[22] Hence, when using any of the irrigating solutions that exhibit good antimicrobial characteristics, bio-mechanical canal preparation using an appropriate technique is equally important for reducing micro-organisms.

Use of rotary instruments has significantly revolutionized the bio-mechanical preparation and has reduced iatrogenic clinical errors for example, ledging, transportation of canal, zipping and sometimes, apical blockage of root canals. ^[23] Hence, both irrigating agents and intra-canal agents act as adjuncts with each other by increasing anti-microbial effectiveness of biomechanical cleaning and shaping. Thus, result in an increase in clinical effectiveness.

Due to unique anatomical localization and isolation, bacterial population within the root canal systems thrive richly and are usually beyond the activity of host defensive systems. The most important bacterial species that has been linked with secondary type of root canal infection is *Enterococcus faecalis*. It has been found to be prevalent as well as persistently present in infections of root canals in up to 24 % to 77 % cases. ^[24]

Nielsen and Baumgartner (2017) have concluded in their study that EndoVac showed more significant results in debridement of root canal at the apical portion of root when compared with irrigation using positive pressure needle. ^[22] Shin et al (2010) also demonstrated that irrigation using EndoVac needles left significantly less amount of debris when compared with irrigation using conventional use of needle. ^[23]

There are multiple factors that can affect efficiency of irrigation using needle which include- diameter of an needle for irrigation, design of irrigating needle, depth of irrigation needle when it is engaged within a root canal and final enlargement size of root canal after completion of preparation. Hence, using a close ended single sided vented needle has been considered as safest design for an irrigating needle.^[24]

Sedgley et al (2005) have recommended the needle to be positioned in close location to canal working length for improvement of canal debridement as well as concomitant replacement of irrigating solution.^[25]

Hence, complete elimination of microbes from within the apical portion of a canal is of primary importance for a successful endodontic therapy.

In contrast to our study findings wherein rotary file instrumentation was found to aid in the best root canal disinfection, Shariq et al (2022) reported with no advantage of rotary instruments with different tapers or use of different irrigating agents i.e., sodium hypochlorite or chlorhexidine. ^[26]

Disinfection remains to be the most important component of debridement in root canals. Due to mechanism of flushing, irrigation helps in removal of microorganisms and remnants of pulpal tissues along with chips of dentin following root canal cleaning and shaping. Irrigation helps in dissolving organic as well as inorganic components in the root canals and has an anti-microbial function that aids in active destruction of bacterial population on directly contacting the canal microbes.

Traditional method involves use of a syringe and an attached needle through which irrigating solutions are introduced within root canals. Boutsioukis et al (2007) concluded in their study that a significant drawback linked with this particular technique for irrigation is lesser flowability of irrigating solution throughout root canal morphology. Highest velocity of irrigation solution streaming is found within needle lumen as well surrounding needle tip, this results in inadequate irrigation during endodontic therapy.^[27] Hence, the disinfection achieved by making use of conventional use of needle is inadequate resulting in increase in the rate of failure of endodontic treatment. In our study also, least reduction in E. faecalis population was noted in group I wherein root canal was irrigated using conventional technique of syringe and needle.

According to Gade et al (2013), when EndoVac irrigation system is used, higher amount of irrigation solution may be delivered in root canals due to its delivery or evacuation needle tip. When its cannulas are inserted within a root canal, constant amount of flowability of an irrigant is delivered within the entire working length of the root canal due to negative intracanal pressure. The EndoVac irrigating system has apically directed negative system of pressure which is capable of removing more amounts of debris at apical 3rd portion of root canal on comparison with conventional method of irrigation by needle that cannot completely remove canal debris during instrumentation. ^[28] In our findings also, the EndoVac irrigation system was found to be more effective in

reducing colony forming units or population of E. faecalis.

Current study reported best control of intra-canal E. faecalis population using rotary endodontic instrumentation. However, in contrast to our study Shuping et al (2000) in their study demonstrated that the rotary endodontic instruments cannot cause full elimination of root canal bacterial populations when sodium hypochlorite (NaOCl) is used as an irrigation medication. ^[29]

CONCLUSION

Elimination of bacterial infection from within root canal is the primary requirement for a successful endodontic treatment. This can be achieved by good instrumentation technique, use of suitable irrigating solution and appropriate obturation. In recent years, there have been numerous studies that have used variety of irrigating medicaments as well as techniques using different types of irrigating needles. In present study, it was found that conventional use of needle and syringe for irrigation purposes reduced colony counts of E. faecalis to a lesser extent when compared to EndoVAc which makes use of laterally situated vent and irrigation while using rotary instrumentation. Root canals which were irrigated during rotary endodontics were found to achieve maximum amount of reduction in E. Facalis colony counts. Hence, it can be concluded from the study that rotary endodontic instrumentation is more capable of achieving reduction in root canal systems.

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