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Original Research Article

COMPARISON OF ANTIBACTERIAL EFFICACY OF VARIOUS ROOT CANAL IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS": AN INVITRO STUDY

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Abstract

Background: The most common cause for the endodontic failure is persistence of microorganisms in root canal system. Enterococcus faecalis is the most frequently isolated species from the cases of failed endodontic therapy. For the complete elimination of bacteria from the root canals, an effective intracanal irrigation regimen is usually required. **Objectives:** Aim of the invitro study is to compare the antimicrobial efficacy of 1.3% NaOCl, 1.3% NaOCl in combination with 15% EDTA, 1.3% NaOCl in combination with Biopure MTAD and 1.3% NaOCl in combination with 2% chlorhexidine solution during root canal irrigation. **Material & Methods:** 52 decoronated root specimens were inoculated with Enterococcus faecalis suspension and incubated for four weeks at 37°C.The specimens were divided in to four groups of 10 each and irrigated with the following irrigation regimen during biomechanical preparation. **Group A-** 1.3% NaOCl, **Group B-** 1.3% NaOCl and 15% EDTA solution, **Group C-** 1.3% NaOCl and Biopure MTAD, **Group D-** 1.3% NaOCl and 2% CHX solution. Root canals were then filled with RTF and circumferentially filed with 15 H files. The canal contents were collected with paper points in to the test tubes containing RTF which were agitated in a vortex mixer. 10 fold serial dilutions were prepared and 5µL of the solution from each tube was then inoculated on BHI agar plates and incubated for 48 hours to obtain bacterial CFU. The results were then subjected to ANOVA and Multiple Comparison Tukey HSD test. **Results:** Irrigation with 1.3% NaOCl and 15% EDTA was effective at killing Enterococcus faecalis followed by 1.3% NaOCl and Biopure MTAD, 1.3% NaOCl and 2% Chlorhexidine, 1.3% NaOCl, Normal saline in decreasing order of efficacy. **Conclusion:** Irrigation with 1.3% NaOCl and 15% EDTA is effective at eradicating the Enterococcus faecalis from radicular dentin.

INTRODUCTION

Maxim states that in endodontics, "it is what you take out of the root canal is important, and not what you put in". While canal preparation is the primary mechanism for removal of canal contents, irrigation serves as a valuable aid in this process. Success of the root canal therapy is dependent on mechanical preparation, irrigation, microbial control and complete obturation of root canals.[1]

Enterococcus faecalis is the most commonly isolated species from the canals of teeth presenting post treatment failure. Enterococcus faecalis is persistent Gram positive cocci that despite being up a small portion of the flora in untreated canals play a major role in the aetiology of periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and is able to survive in the root canal as a single organism or as a major component of the flora. Enterococcus is a facultative anaerobe, possessing the ability to grow in the presence or absence of oxygen. Enterococci can survive very harsh environment including extreme alkaline pH (9.6) and salt concentrations.

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They can grow in the range of 10 – 450c and survive in the temperature of 600c for 30 minutes.[2]

Elimination of microorganisms from the infected root canal is a complicated task. The mechanical action of the instruments alone is not capable of cleaning a root canal satisfactorily owing to the complexity of the internal dental anatomy (apical deltas, lateral canals, accessory canals etc.) because direct contact between instruments and all the walls of the root canal system is not possible.[3]

Antibacterial irrigation solutions may reach canal ramifications and inaccessible areas and permeate completely through the dentinal tubules4. Irrigants are important for the removal of debris and dentinal chips produced during cleaning and shaping. An ideal irrigant should have a broad antimicrobial spectrum and high efficacy against aerobic and facultative microorganisms organized in biofilms, dissolve necrotic pulp tissue remnants, inactivate endotoxin, prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed.[4]

A variety of solutions have been advocated for irrigation during root canal treatment. These include Sodium hypochlorite, EDTA, Normal saline, Chlorhexidine, Hydrogen peroxide, Detergents, MTAD, Ozonated water, Electro chemically activated water etc. No irrigant meets all the requirements of an ideal irrigant

The most commonly used irrigants have shown many limitations in their action such as inability to remove the smear layer, inability to dissolve the organic tissue, incomplete action against bacteria because of the resistance of some strains etc. resulted in the failure of endodontic therapy. The search for a new irrigant has continued for a long time.

Aim of the in vitro study is to compare the antimicrobial efficacy of 1.3% NaOCl, 1.3% NaOCl in combination with 15% EDTA, 1.3% NaOCl in combination with Biopure MTAD and 1.3% NaOCl in combination with 2% Chlorhexidine solution during root canal irrigation.

MATERIALS AND METHODS

Preparation of Teeth

52 extracted single rooted mandibular human first premolar teeth with mature apices were collected. The teeth were soaked in 5.25% NaOCl for 30 minutes to remove residual loose tissue and debris from the root surface. The teeth were stored in distilled water until use. Each tooth was radiographed to confirm the presence of single root canal. The teeth were decoronated perpendicular to the long axis of the root using a rotary diamond disc under continuous water irrigation so as to obtain a root length of 12mm. An access opening was prepared and the pulp was removed with a barbed broach. Working length was determined by visual method for each tooth by using #15k file. The canals were instrumented to a standard apical size of #25 hand k file (Mani) to facilitate bacterial inoculation. Each root was dried and three layers of nail varnish were coated all over the external root surfaces with care not to occlude the root canal entrance.

A customized model was fabricated for each tooth that allowed handling of the tooth during the instrumentation sequence of the experiment by injecting polyvinyl siloxane impression material (Dentsply Maillefer) into aluminium mould. The roots were then mounted in aluminium mould filled with polyvinyl siloxane impression material to the coronal one-third of the root before setting of the impression material so that the impression material was moulded around the root. Each tooth, along with scintillation vial and its corresponding customized tooth model were steam autoclaved by placing them in autoclavable bags for 30 minutes under 15 psi pressure at 121°C. Each tooth was then placed in a sterilized vial, immersed in sterile Brain heart infusion (BHI) broth (Himedia), and sealed. Six teeth stored in sterile Brain heart infusion broth (BHI) without any bacterial inoculation served as negative controls which were examined throughout the experimental period to ascertain the effectiveness of the sterilization procedures. From this stage forward, all samples were processed using strict aseptic protocols.

An inoculum of a 24-hour pure culture suspension of Enterococcus faecalis (ATCC 19433) grown in BHI broth was prepared. Five millilitres of this culture was added to the scintillation vial containing BHI broth and the sterilized tooth. The teeth were incubated with Enterococcus faecalis for 4 weeks under aerobic conditions at 37°C. The media was replenished on every seventh day. Random sampling and Gram`s staining, Catalase test, Haemolysis on blood agar plate, Bile esculin test, Growth on 6.5% Sodium chloride, Tellurite tolerance test were performed to confirm the viability and purity of the Enterococcus faecalis culture.

Grouping of Specimens

After 4 weeks the teeth were removed from the broth and inserted into customised model and the interface between the outer tooth surface and impression material was sealed with cyanocrylate. 52 teeth were divided into 4 experimental groups, one positive control group and one negative control group. 10 teeth were assigned into each experimental group, 6 teeth into positive control and 6 teeth in to negative control group. The irrigation regimen in different groups is as follows

Experimental groups - Group A, B, C, D

- Group A- Irrigation with 1.3% NaOCl,
- Group B- Irrigation with 1.3% NaOCl and 15% EDTA solution,
- Group C- Irrigation with 1.3% NaOCl and Biopure MTAD,
- Group D- Irrigation with 1.3% NaOCl and 2% Chlorhexidine solution.

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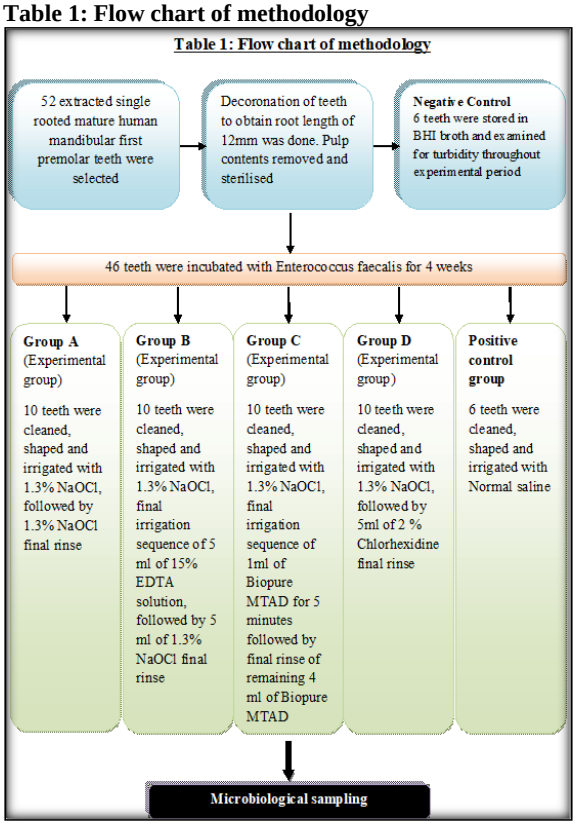
Microbial sample collection

Canals were dried with sterile absorbent paper points and then filled with reduced transport fluid. Sterile #15 H files were placed in to the canals to within 1 mm of working length and the canals were circumferentially filed for 10 seconds. Three consecutive coarse sterile paper points were introduced into the canal to absorb the reduced transport fluid. The paper points were then transferred to the test tube containing the reduced transport fluid. All the collected samples were vortexed in vortex mixer for 10 seconds and 10 fold dilutions were prepared.

Aliquots of 0.1 ml suspension were plated onto BHI agar plates and incubated at 370c for 48 hours. Colony forming units (CFUs) per 1 ml were enumerated.

One-way Analysis of Variance and Multiple comparison Tukey HSD test with significance set at $p<0.05$ was used to analyse the differences in the data obtained.

Table 1: Flow chart of methodology



RESULTS

All the samples in the negative control group showed the absence of turbidity throughout the experimental period and no growth on Brain Heart Infusion agar plates (BHI) whereas all the samples in the positive control group showed the presence of bacterial growth on BHI agar plates. Positive control group (Normal saline) showed the highest mean colony count of Enterococcus faecalis among all the groups which is 389±67. Among the experimental groups, Group A (1.3% NaOCl) showed the highest mean colony count of Enterococcus faecalis which is 220±29 and Group B (1.3% NaOCl and 15% EDTA) showed the least mean colony count which is 135±11. Group C (1.3% NaOCl and Biopure MTAD) and Group D (1.3%NaOCl and 2% Chlorhexidine) showed 157±13 and 200±23 mean colony count respectively.

Statistical analysis of the data using one-way ANOVA test showed significant difference in the antibacterial efficacy of irrigants among the groups. Multiple comparison Tukey HSD test with significance set at $p<0.05$ showed no significant difference in the mean colony count between Group B (1.3%NaOCl and 15% EDTA) and Group C (1.3% NaOCl and Biopure MTAD). Group A (1.3% NaOCl) showed significant difference in the mean colony count of Enterococcus faecalis with Group B (1.3% NaOCl and EDTA), Group C (1.3% NaOCl and Biopure MTAD) and Positive control group (Normal saline). There was no significant difference between Group A (1.3% NaOCl) and Group D (1.3% NaOCl and 2% Chlorhexidine). There is significant difference between the positive control group and all the experimental groups ($p<0.05$).

Among Group B (1.3% NaOCl and EDTA), Group C (1.3% NaOCl and Biopure MTAD) and Group D (1.3% NaOCl and 2% Chlorhexidine) where different combination of irrigants were used Group B showed the least and Group D showed the highest colony count of Enterococcus faecalis. [Table 1]

(Positive Control-Sterile Normal saline, Group A-1.3% NaOCl, Group B-1.3% NaOCl and 15% EDTA solution, Group C- 1.3% NaOCl and Biopure MTAD, Group D-1.3%NaOCl and 2% Chlorhexidine solution). [Table 2]

(Positive Control-Sterile Normal saline, Group A-1.3% NaOCl, Group B-1.3% NaOCl and 15% EDTA solution, Group C-1.3% NaOCI and Biopure MTAD, Group D-1.3%NaOCI and 2% Chlorhexidine solution). [Table 3]

(Positive control-Sterile Normal saline, Group A-1.3% NaOCl, Group B-1.3%NaOCI and 15% EDTA solution, Group C-1.3% NaOCI and Biopure MTAD, Group D-1.3% NaOCl and 2% Chlorexidine solution). [Table 5]

Table 2: Number of Colony Forming Units of Enterococcus Faecalis/Ml (10)

Sample	Group A	Group B	Group C	Group D	Positive control	Negative control
1	2.05	1.31	1.51	1.86	3.98	0
2	2.31	1.41	1.65	2.11	4.58	0
3	1.75	1.15	1.35	1.70	2.89	0
4	1.96	1.25	1.47	1.79	4.38	0

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5	2.76	1.50	1.76	2.39	3.25	0
6	2.35	1.44	1.71	2.19	4.29	0
7	2.25	1.36	1.56	1.96		
8	2.45	1.49	1.73	2.30		
9	2.27	1.39	1.62	2.02		
10	1.88	1.22	1.42	1.75		

Table 3: Mean Colony Count of Enterococcus Faecalis (10)

Groups	N	Mean	Std. Deviation
Positive control	6	3.895	0.677
Group A	10	2.203	0.299
Group B	10	1.352	0.117
Group C	10	1.578	0.139
Group D	10	2.007	0.238
Total	46	2.060	0.839

Table 4: One Way Anova Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	27.772	4	6.943	72.936	0.000
Within Groups	3.903	41	0.095		
Total	31.675	45			

Table 5: Antibacterial Efficacy of Root Canal Irrigants in Decreasing Order

Antibacterial Efficacy	
1	Group B (1.3% NaOCI and 15% EDTA)
2	Group C (1.3% NaOCl and Biopure MTAD)
3	Group D (1.3% NaOCl and 2% CHX)
4	Group A(1.3% NaOCl)
5	Positive control (Normal saline)

Table 6: Hependant Variable: Mean Colony Count of Enterococcus Faecalis Tukey Hsd Test

(1) GROUP	(J)GROUP	Mean Difference(l-l)	Stg.
Conrrol	Group A	1.692*	0,000
	Group B	2.543*	0.000
	Group C	2.317*	0.000
	Group D)	1.888*	0.000
Group A	Control	-1.692*	0.000
	Group B	0.851*	0.000
	Group C	0.625*	0.000
	Group D	0.196	0.618
Group B	Control	2.543*	0.000
	Group A	-0.851*	0.000
	Group C	-0.226	0.483
	Group D	-0.655*	0.000
Group C	Control	-2,317*	0.000
	Group A	-0.625*	0.000
	Group B	0.226	0.483
	Group D	-0.429*	0.027
Group D	Control	-1.888*	0.000
	Group A	-0.196	0.618
	Group B	0.655*	0.000
	Group C	0.429*	0.027

* Indicates-Significant difference exists between the groups
The mean difference is significant at $p<0.05$

DISCUSSION

As no single irrigant meets all the ideal requirements of an irrigant, in the present study combinations of irrigants were tried with the idea of maximising the advantages of the ideal properties of individual irrigants and at the same time minimising their disadvantages.

In the present study 52 teeth were divided into four experimental groups with 10 teeth each and two control groups with 6 teeth each. The teeth were decoronated to obtain a root length of 12 mm. Cementum was not removed to simulate clinical condition. After determination of working length the teeth were instrumented to the apex using a 25 k file to create a patency and facilitate the intracanal contamination procedure.[6] Root canals were inoculated with Enterococcus faecalis for four weeks to allow penetration of the microorganisms into the dentinal tubules.[7] A single microorganism was used to contaminate the root canals to allow for ease of maintaining and accounting for a single species.

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After the completion of incubation period, instrumentation was carried out with Protaper rotary files S1, Sx, S2, F1 and F2 in the four experimental groups and positive control group following the irrigation protocol. Microbial samples were collected with sterile paper points from the root canal.

Various studies reported the use of paper points for collecting microbial samples (Siqueira et al, Dalton etal, and Kuruvilla et al). Paper point cultures of the root canal detected bacteria more frequently than dentin filling cultures on the reamers.[8]

In the present study three coarse paper points were used for collecting the microbial samples. The results of the present study revealed highest mean colony count of Enterococcus faecalis in Group A (1.3% NaOCl alone) among the experimental groups. Statistical analysis using Tukey Multiple comparison revealed significant difference in the mean colony count between Group A (1.3% NaOCl) and Group B (1.3% NaOCl and 15% EDTA), Group A (1.3% NaOCl) and Group C (1.3% NaOCl and Biopure MTAD). There was no significant difference between Group A (1.3% NaOCl) and Group D (1.3% NaOCl and chlorhexidine).

The ineffectiveness of NaOCl to consistently disinfect root canals is in agreement with the results of previous investigations of Sjogren et al (1997), Siqueira et al (1997), and Shuping et al (2000). The lack of increased antimicrobial efficacy may be due to the inability of NaOCl to remove the smear layer, its inability to penetrate in to the dentinal tubules.

Among the experimental groups the mean colony counts of Enterococcus faecalis in Group D (1.3% NaOCl and 2% Chlorhexidine) is less when compared to Group A (1.3% NaOCl). The combined use of NaOCl and Chlorhexidine within the root canal resulted in a greater reduction of microbial flora when compared to the use of NaOCl alone, the results was very much similar with study of Mubssira Shaikh et al (2017).[14]

The results are in corroboration with Vijaykumar et al (2010) and Pallavi Goel et al (2022) where the authors has compared the reduction of Enterococcus faecalis counts in root canals produced by irrigation with Hydrogen peroxide, Sodium hypochlorite, Chlorhexidine and combination of the solutions invitro. This synergistic effects gained by the NaOCl and Chlorhexidine combination include an additive antimicrobial action and better tissue dissolution ability.[9,15]

The mean colony count in the positive control showed that irrigation using sterile Normal saline is unable to render the root canal system free of bacteria and that the bacteria remained viable. The samples from the negative control group showed no signs of turbidity in Brain Heart Infusion broth and absence of growth on BHI agar plates.

In Group B irrigation was done with 1.3% NaOCl followed by 15% EDTA. This was followed by a final rinse of 1.3% NaOCl. This is because according to Marshall GW etal and Habelitz.S et al it is reported that 15% EDTA as a final irrigant at pH 7.3 for 5 minutes produced a 20-30 µm zone of demineralisation. According to Anilkishen et al 10(2008) final irrigation with EDTA showed greater number of adhering Enterococcus faecalis. Demineralisation of dentin exposes collagen creates an ideal substrate for adherence by Enterococcus faecalis (Makinen PL et al). When NaOCl is used as the final irrigant the exposed collagen will be removed and subsequently the number of adhering bacteria will be reduced. group B results are very much similar wity study of Ebtissam M and Al-Madi et al (2019) on NaOCl.[16]

Among the experimental groups Group B (1.3% NaOCl and 15 % EDTA followed by a final rinse of 1.3% NaOCl) showed lowest mean colony count of Enterococcus faecalis. There was significant difference in the mean colony count of Enterococcus faecalis between Group B and Group A (1.3% NaOCl), between Group B (1.3% NaOCl and 15 % EDTA) and Group D (1.3% NaOCl and 2% CHX). There was no significant difference between Group B (1.3% NaOCl and 15 % EDTA) and Group C (1.3% NaOCl and Biopure MTAD). The results are in accordance with Patricia.kho and J. Craig Baumgartner (2006) 18 who compared the antimicrobial efficacy of irrigation with 1.3% NaOCl and Biopure MTAD versus irrigation with 5.25% NaOCl and 15% EDTA.

The effectiveness of 1.3% NaOCl and Biopure MTAD to consistently disinfect the root canals in this study disagrees with the results of Shabahang and Torabinejad. Differences in the results may be attributed to the methodology and microbial sampling procedures followed. The present study used techniques to sample the canal contents immediately after debridement while Shabahang and Torabinejad assessed for turbidity in a growth media after one-week incubation period. Furthermore, they soaked the entire tooth in Biopure MTAD for five minutes after debridement.[24] This part of the methodology was excluded in this study. In the present study the canal lumen of the samples were filled with the irrigants to more closely simulate clinical use.[16]

Torabinejad et al 10 has compared the effectiveness of MTAD and NaOCl (5.25%) using the zone of inhibition technique and discovered their similar antibacterial action against Enterococcus faecalis. Tay etal has compared MTAD, NaOCl (1.3%) and the consecutive use of irrigants. Their results confirmed that MTAD was most effective irrigant in eliminating Enterococcus faecalis. However, they found no difference between NaOCl and NaOCl in combination with MTAD.

Mohammed Asna Ashari et al 10 (2009) has compared the antimicrobial effect of MTAD, NaOCl (5.25%) and their combination on endodontic pathogens and found that antimicrobial effect of the mixture was less than MTAD or NaOCl alone. Both NaOCl and MTAD are strong antimicrobials when used independently. NaOCl when used in

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combination with MTAD, NaOCl reduces the antimicrobial power of MTAD. This is because of the oxidation of MTAD by NaOCl similar to the peroxidation of tetracycline with reactive oxygen species which was confirmed in Tay's study12, Vivek Kapoor.[17] This could be the reason for the reduced antimicrobial activity in Group B (1.3% NaOCl and Biopure MTAD). There have been reports that antioxidant such as ascorbic acid rinse following NaOCl irrigation will remove the remnants of hypochlorite.[9,14]

Although an irrigant can penetrate in to the dentinal tubules it does not mean that the concentration of the irrigant alone is sufficient to kill all types of bacteria present. Bacteria may remain viable in the dentinal tubules at greater distances from the pulp. Disinfection of the root dentin by chemomechanical preparation alone is questionable.

Bacteria deep in the dentinal tubules are apparently protected from instrumentation and irrigation making their removal or eradication difficult. Microorganisms may be eliminated or rendered harmless by entombing them through complete obturation of the canal space after chemo-mechanical root canal preparation. Although the consequences of microbes remaining in the dentinal tubules after root canal treatment is not clear the main goal of root canal treatment is still the elimination of microorganisms. The efficacy of other irrigants and more effective irrigation delivery system needs further research.

CONCLUSION

Within the limitations of the study, following conclusions were drawn

1. Irrigation with Normal saline was unable to eradicate Enterococcus faecalis from the root canals as expected.
2. Irrigation with Sodium hypochlorite alone without any combination of irrigants was less effective in eradicating Enterococcus faecalis.
3. Among the different combinations of irrigants evaluated (2% Chlorhexidine solution, 15% EDTA solution and Biopure MTAD with 1.3% NaOCl) 2% Chlorhexidine solution in combination with 1.3% NaOCl showed least antibacterial efficacy in eradicating Enterococcus faecalis.
4. When 1.3% NaOCl and 15% EDTA, 1.3% NaOCl and Biopure MTAD was compared 1.3% NaOCl and Biopure MTAD combination was less effective in eradicating Enterococcus faecalis.
5. Among all the irrigants evaluated 1.3% NaOCl in combination with 15% EDTA showed the highest antibacterial efficacy in eradicating Enterococcus faecalis from the root canals. Hence this can be routinely used as an effective root canal irrigation regimen against Enterococcus faecalis in endodontic failure cases.

Although the results of the present study indicate that 1.3% NaOCl and 15% EDTA was the most effective irrigation regimen further studies are needed to determine the effect of these findings in clinical settings.

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