Dentistry
ISSN: 2161-1122

Srirekha et al. Dentistry 2018, 8:7
DOI: 10.4172/2161-1122.1000502

Research Article Open Access

Comparative Evaluation of Chlorhexidine and its Combination with Chitosan as Intracanal Medicaments on Enterococcus faecalis in Endodontic Retreatment

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Abstract

Aim: The aim of this study was to evaluate the efficacy and synergistic effect of chitosan in combination with chlorhexidine gel and chlorhexidine alone as intra canal medicaments in retreatment cases.

Materials & Methods: Thirty single rooted teeth indicated for root canal retreatment were selected. After gutta percha (GP) removal, a pre-treatment sample (S1) was taken. Following which cleaning and shaping using sodium hypochlorite and EDTA was done and post-instrumentation sample (S2) was collected. The teeth were randomly divided into two groups Group I (n=15): 2% chlorhexidine gluconate gel Group II (n=15): 2% chlorhexidine gluconate gel and 2% chitosan (1:1). Post medication sample (S3) was taken after seven days and sent for quantitative PCR evaluation for E. *faecalis*. The data was statistically analyzed using Mann Whitney U test and Kruskal Wallis test ($p<0.05$).

Results: The results from Kruskal Wallis test indicate comparison of bacterial counts in group 1 and group2 for S1, S2 and S3 showed bacterial reduction count from S1 to S2. The bacterial count difference between S1- S3 was better in group 2 compared to group 1. However, it was not statistically significant ($p<0.213$).

Conclusion: Cleaning and shaping and irrigation along with use of intracanal medicament resulted in decrease in the mean *E. faecalis* in both the groups. 2% chlorhexidine gluconate gel in combination with chitosan performed better than chlorhexidine alone against *E. faecalis*, However, it is not statistically significant. ($p<0.213$).

Keywords: Retreatment; Polymerase chain reaction; ProTaper retreatment files Chlorhexidine gluconate; Chitosan

Introduction

Bacteria remaining within the root canal system are a significant factor in endodontic failures. Hence, retreatment of endodontically treated teeth is a major challenge to clinicians. *Enterococcus faecalis* has been most frequently identified in 12% to 77% of the cases in canals of root-filled teeth with periapical lesions either by culture or molecular methods [1]. Molecular analyses have revealed the presence of uncultivable or difficult-to-grow bacteria in infected root canals, providing the opportunity to obtain a significant amount of new information on endodontic microbiota [2]. Recent advances in cellular and molecular biological methods revealed real- time quantitative Polymerase Chain Reaction (PCR) is 10-100 times more sensitive [3]. Cleaning and shaping effectively reduce microbiota but these procedures do not completely eliminate bacteria in the lateral and accessory root canals, isthmi, and apical deltas.

Intracanal medicaments are used as an antibacterial agent to eliminate residual bacteria in a root canal after instrumentation and irrigation, to render any remaining canal content inert [4]. Chlorhexidine (CHX) is a potent antimicrobial frequently used in endodontics [5]. It is a broad spectrum antibacterial agent that is effective against both *E. faecalis* and *Candida albicans.* In addition to its immediate action on bacteria, chlorhexidine can be adsorbed onto and subsequently released from dental tissues, resulting in substantive antibacterial activity or "substantivity" [6]. Chitosan is an amino polysaccharide biopolymer, which displays excellent biocompatibility, physical stability and processability. It has widely been used in the area of dentistry as a bioadhesive; viscosity-enhancer; for prolonged drug release in buccal cavity; permeabilizer; antimicrobial; anti-adhesive; anti-cariogenic; for treatment of periodontal diseases/oral candidiasis/ tooth mobility and reduction of plaque formation [7]. However its role in endodontics in vivo has not been subjected to adequate scrutiny. The in vivo evaluation of synergistic effect of chitosan and its combination with CHX as intracanal medicament against the resistant bacteria like E,faecalis will have important clinical implications in nonsurgical endodontiv retreatment.

Hence, the aim of this clinical study is to evaluate the antimicrobial efficacy of chlorhexidine and its combination with chitosan as intracanal medicament in response to *E. faecalis* in retreatment cases.

Materials and Methods

Thirty patients between 19-65 years of age requiring root canal retreatment were selected for the study. All selected teeth were single rooted which were symptomatic or asymptomatic and had history of root canal treatment and showed periapical radiolucency upto 4mm. Ethical clearance was obtained from the institutional ethical committee. All patients were explained about the treatment procedure and informed consent was taken.

The selected tooth was isolated with a rubber dam. The crown and surrounding rubber dam were disinfected with 30% hydrogen peroxide for 30s followed by vigorous swabbing of 3% sodium hypochlorite solution. Subsequently 5% sodium thiosulfate was used to inactivate the disinfectant. Access preparation was done with a sterile Endo access

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Received July 16, 2018; **Accepted** July 26, 2018; **Published** July 28, 2018

Citation: Kapadia M, Srirekha A, Lekha S, Savitha B, Vijay R, et al. (2018) Comparative Evaluation of Chlorhexidine and its Combination with Chitosan as Intracanal Medicaments on Enterococcus faecalis in Endodontic Retreatment. Dentistry 8: 502. doi:10.4172/2161-1122.1000502

bur (Dentsply Maillefer, Ballaigues, Switzerland) until root canal filling was exposed. On exposing the previous root filling, the second stage of disinfection was done for the operative field by vigorous swabbing of 3% sodium hypochlorite solution. Subsequently 5% sodium thiosulfate was used as a neutralizer.

Gutta percha (GP) was removed with Protaper Retreatment files in sequence of D_1, D_2 and D_3 (Protaper universal, Dentsply, India) as per the manufacturer's instructions and no chemical solvents were used in retrival of gutta percha. A periapical radiograph was taken to check the complete removal of GP. First Sample (S1) contained retrived GP, dentinal shavings and paper point which were transferred aseptically to tubes containing transport media. Working length was measured by preoperative radiographs and # 15 file was inserted into the root canal 0.5 mm short of the root apex and working length was confirmed using electronic apex locator (Root ZX mini, J Morita Corp, Tokyo, Japan).

Canals were irrigated using 5 ml 3% sodium hypochlorite and 17% EDTA during instrumentation. To inactivate sodium hypochlorite, 3 ml, 5% sodium thiosulphate was used followed by flushing with saline. After drying the root canal with sterile paper points a second root canal sample (S2) was taken. Patients were randomly divided into two study groups:-

Group I (n=15): 2% chlorhexidine gluconate gel

Group II (n=15): 2% chlorhexidine gluconate gel and 2% chitosan (Everest Biotec, Bangalore) (1:1)

Intracanal medicaments in Group I was placed in gel form and in Group II in paste form. The access was then sealed with sterile cotton pellet and Resin modified Glass Ionomer Cement.

Patients were recalled after 7 days and all restorations were checked for integrity and dislodged restorations were excluded from the study. Restoration was removed using sterile bur and residual intra canal medicament was flushed out with saline and the canal walls were lightly filed with no. 30 H file.

In Group I: 2 ml of 3% Tween 80 was used to neutralize chlorhexidine gluconate, over a period of 5 minutes followed by irrigation with sterile saline. In Group II: along with 2ml of 3% Tween 80, serial dilution using saline was used to neutralize the combination.

To evaluate residual burden of *Enterococcus faecalis* microorganism post medicament sample (S3) was taken. After sample collection root canal treatment was completed. None of the patients in this study needed intervention during treatment due to flare up.

Quantitative Real time Polymerase Chain Reaction

Total genomic DNA from the bacteria was isolated by N- Cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method. The quantity of the isolated DNA was checked in UV-VIS spectrophotomer (Vivaspec Biophotometer, Germany). From the stock 1 µl DNA was mixed with 49 µl sterile distilled water to get 50 times dilution. The A260/A280 ratio was recorded to check the purity of DNA preparation.

Real-time PCR was performed using a StepOne, Applied Biosystems USA. Real-time PCR was carried out with an initial incubation of 10 min at 95°C followed by 35 cycles consisting of denaturing at 95°C for 10 seconds; annealing at 60°C for 5 sec followed by amplification at 72°C for 30 seconds. Amplification and detection were carried out in optical-grade 48 well plates in an ABI Prism Sequence Detection System. After amplification, a melting curve analysis was performed to determine the specificity of the PCR products. Threshold cycle (Ct) analysis of all samples were either set at 0.5 relative fluorescence units or left to automatic detection by the system. Quantification analysis was performed using StepOne Plus software.

Results

Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables etc.

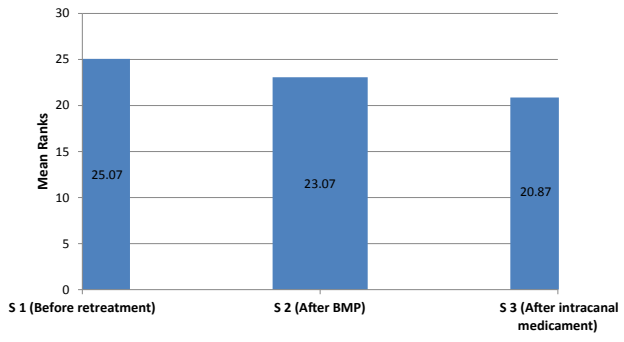
Results in Table 1 indicate that the percentage reduction of bacteria in samples dropped from S1 to S2. Decrease in microbial load was seen from S2-S3 at the end of 7 days in both groups. The comparison of bacterial counts (Mean rank) in group 1 and group 2 at different time interval using Kruskal Wallis test is given in Graph 1 and 2

The results show that the bacterial count difference of group 1 and group 2 is 13.50 and 17.50 respectively (Table 2). However, it is not statistically significant ($p<0.05$).

Discussion

Endodontic failures can be attributable to inadequacies in shaping, cleaning and obturation, and also reinfection of the root canal system when the coronal seal is lost after completion of root canal treatment [8]. To increase the efficiency of instrumentation, root canal irrigating solutions and intracanal medicaments are used to eliminate the bacteria from the root canals [9]. This can be ascribed to the usual inability of instruments and irrigants in cleaning and disinfecting anatomical variables, which are very common in the apical portion of the root canals [10,11].

In the present study the results showed a reduction in microbial load in both groups. In Group 1 mean rank for S1, S2 was 25.07, 23.07 (Graph 1) and percentage reduction of bacterial load between S1-S2 was 38.40%, (Table 1) while group 2 showed the mean rank for S1, S2 was 26.33, 23.03 (Graph 2) and the percentage reduction of bacterial load between S1-S2 being 43.15% respectively (Table 1). Various clinical studies that have reported that chemomechanical procedures reduce microorganisms in the root canal system [12,13] Siqueira et al. reported that 4% NaOCl was significantly more effective than saline solution in disinfecting root canals inoculated with *E. faecalis* [14]. Shuping et al. showed after instrumentation with rotary NiTi instruments along with NaOCl irrigation, 61.9% of canals were



Group 1: Chlorhexidine.

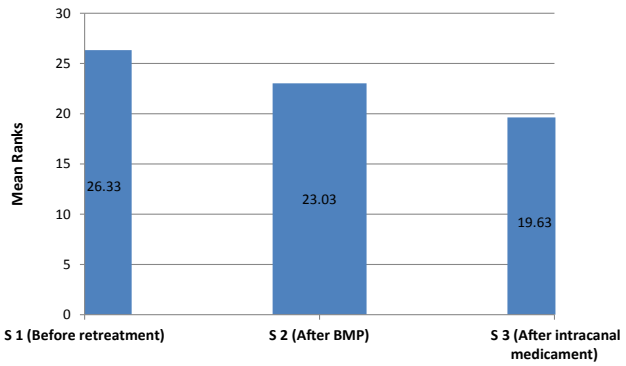
Graph 1: Comparison of bacterial counts (Mean rank) in group 1 at different time interval using Kruskal Wallis test.

Number of Groups	S1-S2	S2-S3	S1-S3
Group 1	38.40%	32.54%	70.94%
Group 2	43.15%	12.27%	55.42%

Table 1: Percentage reduction in group 1 (chlorhexidine) and Group 2 (chlorhexidine and chitosan).

rendered bacteria-free [15]. Although the protocol used in this study reduced *E. faecalis* levels, the bacterium was not totally eliminated from the canal. This is because in spite of the thorough instrumentation tissue remnants can be localized in isthmuses, irregularities, dentinal tubules and lateral canals, which very often remain unaffected by instruments and irrigants [16]. In Group 1 mean rank for S3 was 20.87 (Graph 1) and percentage reduction of bacterial load between S2-S3 and S1-S3 is 32.54% and 70.94% respectively (Table 1). Similar results were found in a clinical study which showed 78% negative cultures after 7 days [17] Chlorhexidine has a broad spectrum antimicrobial effect targeting both gram positive and gram negative microorganisms [18]. It has marked effect against resistant microorganisms in the root canal such as *E. faecalis*, anaerobic bacteria and *Candida albicans* [19]. A study inferred that 2% CHX gel was able to clean the root canal walls and their anatomic complexities effectively because of the viscosity of the gel, which promoted a better mechanical cleansing of the root canal, better removal of dentin debris and the remaining tissue. In addition, 2% CHX gel has good antimicrobial property which can potentially disinfect the dentinal tubules and anatomical complexities during instrumentation as compared to solution form [20].

The results for Group 2 showed that the mean rank for S3 is 19.63 (Graph 2) and the percentage reduction of bacterial load between S2-S3 and S1-S3 is 12.27% and 55.42% respectively (Table 1). Combination of irrigants or medicaments decreases the development of resistant bacterial strains and produces synergistic effect, leading to long lasting antimicrobial action and sustained release of medicaments [21]. The spectrum of antimicrobial activity of chitosan and its derivatives extends to include filamentous fungi, yeasts and bacteria, being more active against gram-positive than gram-negative bacteria [22]. Combination of two medications may produce additive or synergistic effect whose antimicrobial action may last longer and also sustain the release of medicaments [23]. On intergroup comparison, the bacterial count difference between S1 and S3 of group 1 and group 2 is 13.50 and 17.50 respectively (Table 2). This result showed that Group 2 performed better than group 1, however there is no statistically significant ($p<0.213$) decrease in microbial count.



Group 2: Chlorhexidine and chitosan.

Graph 2: Comparison of bacterial counts (Mean rank) in group 2 at different time interval using Kruskal Wallis test.

Groups	No. of samples	Mean rank
Group 1: Chlorhexidine	15	13.50
Group 2: Chlorhexidine + chitosan	15	17.50
Z value	-	1.244
P value	-	0.213

Table 2: Comparison of bacterial counts difference (Mean ranks) {before retreatment (S1)- after placing intracanal medicament (S3)} among both the groups using Mann Whitney U test. Z value (Standard deviation) P value (Statistical significance).

An *in vitro* study was conducted to investigate the antimicrobial effectiveness of 2% CHX gel, 2% chitosan gel and their combination against *Candida albicans* and *E. faecalis*. It was established that release of chlorhexidine with chitosan was better than plain chitosan alone, combination of the two showed maximum inhibitory zone for *Candida albicans* and *E. faecalis*. This study advocated that 2% chlorhexidine gel in combination with 2% chitosan had better antimicrobial efficacy than either of them alone [23].

Conclusion

Within the limitations of this in vivo study the following conclusion can be drawn: Cleaning and shaping (S1-S2) and irrigation along with use of intracanal medicaments like CHX and its combination with chitosan resulted in reduced mean *E. faecalis* load in both the groups, however it was not statistically significant. Intergroup comparison showed 2% CHX in combination with chitosan performed better compared to CHX as an intracanal medicament when used against *E. faecalis*, however it was not statistically significant ($p<0.213$).

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