Efficacy of Chlorhexidine Intracanal Medicament on Periodontal Healing in Concomitant Endodontic-Periodontal Disease with Communication: A Randomized Clinical Trial

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ABSTRACT

Background: Chlorhexidine has been extensively used in various periodontal and endodontic infections. However, its use in combined endo-perio lesion has not been defined clearly. The aim of this study was to evaluate the efficacy and synergistic effect of chlorhexidine intracanal medicament with combination of comprehensive periodontal therapy in endodontic periodontal lesions.

Materials and Methods: The patients were randomly assigned group 1 and group 2. Both the groups were treated by phase 1 and phase 2 periodontal treatments while group 1 patients received conventional root canal treatment and group 2 patients were treated by chlorhexidine intracanal medicament for 6 months. The primary outcome measures are probing pocket depth, relative attachment level and bleeding on probing.

Results: Both groups showed improvement on primary outcome measures. However, group 2 showed significant more reduction in probing pocket depth (PPD) and bleeding on probing (BOP) and gain in relative attachment level (RAL) after complete 6 months treatment with chlorhexidine. Postoperative measurements from group 1 and group 2 showed reductions in mean PPD of 2.63±0.98 and 3.57±1.13 mm, mean BOP reduction of 76.78±31.41 and 87.54±22.35 and mean RAL gains of 2.42±1.27 and 3.42±0.94 mm, respectively.

Conclusion: 2% CHX gel used as an intracanal medicament along with periodontal treatment demonstrated more favourable treatment response in terms of periodontal healing.

Keywords: Chlorhexidine, endodontic treatment and intracanal medicament.

I. INTRODUCTION

The interrelationship between pulp and its periodontium lies in the embryological development as both are derived from common mesodermal source [1]. The primary cause for development of endodontic-periodontal disease is the complex microbial flora [2] and similar microorganisms has been diagnosed between them [3], [4].

Microorganisms from the periodontal lesion may re-invoke completely disinfected the root canal system and vice versa [5]. It is advocated to initiate the periodontal treatment while root canal is still medicated with intracanal medicament to prevent cross contamination of bacteria in either direction [2].

Selection of intracanal medicament which can be used as an antimicrobial agent within root canal system and may diffuse on external root surface to promote periodontal healing is an important aspect. 2% chlorhexidine (CHX) gel used as an intracanal medicament has shown effective broad spectrum antibacterial activity within the root canal system and diffusion from root canals to external root surface [6].

The clinical study of [7] demonstrated significantly improved periodontal healing in concomitant endodontic periodontal lesions without apical communication when 2% CHX gel was used as an intracanal medicament placed in the coronal portion of an adequately treated root canal system without jeopardize apical seal. They suggested that these findings could be attributed to sustained inhibition of bacterial growth and early bacterial re-colonization of the treated area during periodontal healing due to local delivery of intracanal medicament on external root surface.

So, it was hypothesized that in apically communicating endodontic and periodontal lesions when using intracanal
medicament would also have better healing response of periodontium as compared to conventional endodontic treatment along with periodontal therapy.

So far no randomized clinical trial has been conducted to observe the effect of intracanal medicament in treating these cases. Thus the aim of the study was to evaluate the efficacy of 2% CHX intracanal medicament used as an adjunct to periodontal therapy on clinical healing of periodontal tissues in the concomitant endodontic periodontal lesions with apical communication.

II. MATERIALS AND METHODS

A. Study Population and Experimental Design

This study was designed as a prospective randomized double blind controlled clinical trial and conducted in Department of Periodontics and Oral Implantology in collaboration with Department of Conservative Dentistry and Endodontics. The study protocol was carried out in accordance with the ethical standards outlined in the Helsinki declaration 1975, as revised in 2013. The protocol was approved by ethical committee (IEC/2014/115) and registered as (ClinicalTrials.gov as NCT02627326). Participants were informed about the procedure and written consent was obtained for the same. Total sixty patients meeting the inclusion criteria were enrolled.

The inclusion criteria included systemically healthy patients with chronic periodontitis [8] of age between 18-55 years, having ≥ 20 teeth, and having at least one tooth with concomitant endodontic periodontal lesion with communication [9] diagnosed by experienced endodontist on the basis of clinical and radiographic presentation which included wide base pocket, deep probing pocket depth, non-vital tooth (by pulp sensibility testing) with periapical radiolucency, radiographic alveolar bone (marginal bone) destruction with apical communication.

Patients were excluded if they were presented with 1) acute symptoms; 2) systemic illness known to affect the periodontium or outcome of periodontal therapy; 3) on medications such as corticosteroids or calcium channel blockers, which are known to interfere with periodontal wound healing or patient on long term NSAID therapy or bisphosphonates; 4) allergic to medication (CHX, local anaesthetic, antibiotics, NSAID); 5) pregnant or lactating females; 6) smokers (current and past) and tobacco chewers; 7) teeth with grade 3 mobility, root resorption, fractured/perforated roots, unrestorable teeth, previously root canal filled and abutments; 8) developing permanent tooth; 9) aggressive periodontitis; 10) history of periodontal treatment within 6 months prior to the study; 11) more than one consecutive endo-perio involved teeth.

Patients were randomly allocated to the treatment groups according to a computer-generated randomization table. Patients were not aware of the treatment protocol at the time of group allocation. From each patient only one endo-perio involved tooth was selected (Fig. 1).

Group 1 (n = 30 teeth/30 patients), endodontic treatment (ET) and full mouth scaling and root planing (SRP) were performed simultaneously and obturation was done within 10 days. After 3 months of completion of endodontic therapy, periodontal surgical treatment in the form of open flap debridement (OFD) was performed.

Group 2 (n = 30 teeth/30 patients), endodontic treatment (ET) and full mouth scaling and root planing (SRP) were started simultaneously. After biomechanical preparation, 2%CHX intracanal medicament was placed in root canal for 3 months. After 3 months, open flap debridement (OFD) was performed in the respective tooth and medicament was changed and placed further for 3 months. Obturation was done after 3 months of OFD.

B. Clinical Procedure

All the patients at the initial visits underwent full mouth scaling and root planing using ultrasonic scalers (Suprasson P5 Booster; Satelec, Merignac Cedex, France) and hand instruments (Hu-Friedy, Chicago, IL) which was completed in two to three sessions along with education and motivation to maintain the health of periodontium. Simultaneously along with SRP, endodontic treatment was initiated using a standardized protocol.

In group 1, at first appointment all the canals were negotiated, cleaned and filled with calcium hydroxide (Roth P5 Booster; Satelec, Merignac Cedex, France) and hand instruments (Hu-Friedy, Chicago, IL). At the second appointment at least after one week, the calcium hydroxide was removed and obturation was performed using a lateral condensation technique with gutta-percha and zinc oxide eugenol sealer and permanent filling was placed in the form of silver filling.
amalgam.

In group 2, after cleaning and shaping, 2% CHX gel (2% CHX digluconate, methylparaben, hydroxyethyl cellulose, and deionized water, pH 7, 2% Clorexoral gel, Biodinamica Quimica E Farmaceutica, Ibipora, PR, Brazil) was placed in the root canal up to apex using 27 gauge endodontic syringe (Monoject, Sherwood Davis & Geck, St. Louis, MO) and tooth was coronally sealed with glass inomer cement (Version 2, Shofu, Kyoto, Japan). The intracanal medicament was replaced every month and obturation was done 3 months after OFD.

After 3 months of commencement of ET, OFD was performed by investigator who was blinded for the group allocation for ET in both the groups. After achieving local anaesthesia (2% lidocaine with 1:80,000 epinephrine), intracrevicular incision were made and full thickness mucoperiosteal flaps were reflected including neighbouring teeth. Meticulous defect debridement and root planing was carried out using area specific gracey curettes and scalers. After debridement and irrigation with normal saline, mucoperiosteal flaps were repositioned and secured by using 3-0 non absorbable black silk surgical suture. Post-operative instructions were given and sutures were removed after one week (Fig. 2).

![Clinical procedure in tooth](image)

Fig. 2. Clinical procedure in tooth lower right first molar from initiation of endodontic treatment to open flap debridement at 3 months and postoperative recording at 9 months from baseline in CHX group. (a) Preoperative PPD measurement with stent at baseline. (b) Preoperative radiograph showing endo perio communication at baseline. (c) Open flap debridement at 3 months. (d) Sutures placed. (e) Postoperative PPD measurement with stent at 9 months from baseline. (f) Postoperative radiograph at 9 months from baseline.

C. Assessment of Treatment Outcome

The authors of the accepted manuscripts will be given a copyright form and the form should accompany your final submission. Periodontal parameters included: plaque Index (PI) [10] and gingival Index (GI) [11] measured on 4 surfaces of all teeth (mesiobuccal, buccal, distobuccal, lingual) were recorded by first investigator (AS). A calibrated 15 mm periodontal probe (PCP-UNC, HuFriedy, Chicago, IL, USA) was used to measure probing pocket depth (PPD), relative attachment level (RAL) and relative gingival marginal level (RGML) at six line angles (mesiobuccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) of endo perio involved tooth. Bleeding on probing (BOP) was recorded and calculated in percentage. Parameters were measured with customized stents which were made with bioplastic sheet.

Periotest (Medizintechnik guldien, bensheim, germany) was used to measure tooth mobility (TM). Determination of the intraexaminer reproducibility was done by performing double clinical periodontal data recording on 10 patients after 48 hours. Assessment of the mean difference in the scores indicated good intraexaminer agreement (kappa value 0.76 and 0.83 for PD and CAL respectively).

Radiographs with paralleling cone technique were taken with standardized exposure parameters (70kvp, 3.5mAs, and 0.2 seconds) using dental x-ray machine (Runyes X-ray, China). Radio-opaque appearance of gutta percha point upto base of pocket or apex on developed radiograph further verified endo-perio communication.

The radiographs were assessed to observe periapical healing and bone level determination at each follow up, using the software (US National Institutes of Health, Bethesda, MD, USA), a parallel straight line to the root surface was drawn over the digital image to measure the distance between CEJ and the most apical bone level. When the CEJ was not detectable because of the presence of dental restorations, the distance was measured from the apical margin of the restoration. Measurements of bone level were evaluated. Inter-rater reliability score was 0.89 showing a good agreement between the two examiners.

III. Statistical Analysis

Sample size was calculated and a minimum sample of 27 patients per group was required by assuming effect size $= 0.8$ with power $\geq 0.80$ and $P < 0.05$. To compensate for the expected attrition in patient pool over time, a decision was made to enrol 60 patients (30 patients in each group).

The Shapiro-Wilk test showed a non-normal distribution of data. Intragroup and intergroup comparisons were analysed by Wilcoxon signed rank test and Mann-Whitney U test respectively. All statistical analysis was two tailed with significance level at 0.05.

IV. Results

Out of 60 patients (60 teeth) initially recruited, total 47 patients/ 47 teeth (Group 1: n=23 patients/23 teeth and Group 2: n=24 patients/24 teeth) completed the treatment protocol and responded to endodontic and periodontal treatment with definite evidence of clinical and radiographic healing. Thirteen patients (group 1 = 7, group 2 = 6) were dropped out at different stages.

Table I depicts demographic and clinical data for each group and no statistical significance difference was detected in all the variables at baseline measurements between the group 1 and group 2 ($P > 0.05$).
Table II represents intergroup comparison of the improvement in periodontal parameters. Statistically significant more PPD reduction, reduced number of deepest sites with BOP and reduction in tooth mobility (P < 0.05) were observed in group 2 as compared to group 1 after 3 months of ET and SRP. At 6 and 9 months of endodontic therapy (3 and 6 months after periodontal surgery respectively) group 2 (P < 0.05) showed significantly more PPD reduction (mean and deepest site), gain in CAL (mean and deepest site), GI reduction (mean and deepest site) and less sites with BOP (at deepest site) as compared to group 1. Significant gain in bone level in group 2 (P < 0.05) was also observed in group 2 at 9 months of follow up.

V. DISCUSSION

This randomized clinical trial observed the effect of 2% CHX gel intracanal medicament on periodontal healing in concomitant endodontic and periodontal lesions with apical communication. In order to predictably achieve bacteria free root canal systems, use of intracanal medication is recommended in endo perio cases, particularly if the dressing is replaced at regular intervals [9]. Moreover, providing periodontal treatment while the root canals are still medicated creates the most unfavourable environment for bacterial survival/bacterial colonization [12], [13].

Selection of intracanal medication which can be used as an antibacterial agent within root canal system and may promote periodontal healing is an important aspect. 2% CHX gel has been shown to be effective as an antibacterial agent within the root canal system and through diffusion into periodontal ligament can improve periodontal healing [14]. Efficacy of CHX is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls [15] thereby altering the cells osmotic equilibrium [16]. CHX gel was used in this study as an effective broad spectrum antibacterial agent within the root canal system. It also prevents the adherence of Porphyromonas gingivalis to epithelial cells [17]. Deep periodontal pockets with continuous alveolar bone loss are associated with the presence of Porphyromonas gingivalis even after providing periodontal treatment [18], [19]. CHX has shown greater antibacterial substantivity against intracanal Enterococcus faecalis up to a period of 15 days inside dentinal tubules. However, it loses its antibacterial property if used for long periods, and no antibacterial activity was exhibited after 30 days of contact [20]. Thus CHX gel was replaced with fresh CHX gel every month in the present study. The substantivity of CHX has been reported maximum up to 12 weeks [21] and minimum for 4 weeks [22]. Furthermore, CHX gel offers several advantages over the CHX solution like its viscosity keeps the active medicament in close contact with root canal surface and dentinal tubules. It eliminates the need for the use of root canal lubricant and also reduces smear layer formation [23], [24].

This study observed significantly more reduction in PPD, GI, BOP and gain in CAL and bone level in CHX group as compared to control group after 3 months and 6 months of periodontal surgery. These findings could be attributed to sustained inhibition of bacterial growth and prevention of early bacterial re-colonization of the treated area during periodontal healing due to continuous diffusion of CHX for 6 months on external root surface through communication pathways. The results of our study are also in accordance with an interventional study conducted by [7], on non-communicating concomitant endodontic periodontal lesions, they also reported better periodontal healing in terms of reduced PPD and CAL gain at the 6 months of follow up period. Present study being conducted on communicating lesions, included all pathways of communication along with apical foramen could have acted as a major pathway for diffusion of CHX intracanal medicament to periodontium. Reference [25] reported in their clinical and microbiological study that periodontal pocket can be repopulated by pathogenic bacteria Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Prevotella intermedia following SRP and even after periodontal surgery and has been considered as a risk factor for recurrence of disease. OFD was performed as a periodontal surgery procedure rather than regenerative periodontal surgical treatment as severe bone loss occurred in communication cases resulted in mainly non contained defects.

In the present study, CHX medicament in the canal space may also be beneficial in promoting periodontal healing during post-operative periods because of sustained release diffusion, it acquired a longer period of substantivity on the external root surface and prevents the re-colonization of periodontal pathogens. Several models have been proposed in literature for the study of the diffusion of calcitonin [26], sodium hypochlorite [27], intracanal CHX and calcium hydroxide [20] into the dentine and on external root surface and the pH alterations that they produce. Furthermore, [14] and [6] have demonstrated antibacterial antimicrobial activity of CHX intracanal medicament on external root surface against anaerobic and aerobic bacteria. However, precaution should be taken while using CHX, as cytoxic properties on gingival fibroblasts and hypersensitive and allergic reactions have been reported in literature.
TABLE II: COMPARISON IN IMPROVEMENT (Δ) IN PERIODONTAL PARAMETERS BETWEEN GROUPS 1 AND 2

| Site specific parameters | Group 1 (mean±SD) (baseline-3 months) | Group 2 (mean±SD) (baseline-3 months) | P value | Group 1 (mean±SD) (baseline-6 months) | Group 2 (mean±SD) (baseline-6 months) | P value | Group 1 (mean±SD) (baseline-9 months) | Group 2 (mean±SD) (baseline-9 months) | P value |
|--------------------------|--------------------------------------|--------------------------------------|---------|--------------------------------------|--------------------------------------|---------|--------------------------------------|--------------------------------------|---------|
| Δ GI (mean)              | 0.90±0.66                            | 0.60±0.50                            | 0.040   | 1.14±0.47                            | 1.30±0.46                            | 0.439   | 1.52±0.42                            | 1.47±0.52                            | 0.061   |
| Δ GI (deepest)           | 0.62±0.64                            | 0.50±0.58                            | 0.308   | 1.34±0.57                            | 1.30±0.52                            | 0.047*  | 1.47±0.51                            | 1.37±0.49                            | 0.049   |
| Δ GI (mean)              | 0.60±0.50                            | 0.50±0.58                            | 0.780   | 1.03±0.36                            | 1.37±0.57                            | 0.020*  | 1.62±0.57                            | 1.04±0.36                            | 0.500*  |
| Δ GI (deepest)           | 0.50±0.58                            | 0.50±0.58                            | 0.780   | 1.03±0.36                            | 1.37±0.57                            | 0.020*  | 1.62±0.57                            | 1.04±0.36                            | 0.500*  |
| Δ GM (%) (mean)          | 45.60±36.0                           | 39.62±48.20                          | 0.075   | 59.08±32.08                          | 81.25±25.67                          | 0.005*  | 76.78±31.4                           | 87.54±22.3                           | 0.393   |
| Δ GM (%) (deepest)       | 9                                   | 9                                    | 0.795   | 59.08±32.08                          | 81.25±25.67                          | 0.005*  | 76.78±31.4                           | 87.54±22.3                           | 0.393   |
| Δ Δ PD (mm) (mean)       | 0.12±0.38                            | 0.11±0.26                            | 0.737   | -0.40±0.42                           | -0.46±0.60                           | 0.714   | -0.50±0.53                           | -0.48±0.60                           | 0.567   |
| Δ Δ PD (mm) (deepest)    | -0.13±0.45                           | -0.25±0.44                           | 0.385   | -0.73±0.61                           | -0.79±0.77                           | 0.981   | -0.78±0.87                           | -0.91±0.88                           | 0.761   |
| Δ Δ CAL (mm) (mean)      | 12.75±5.41                           | 9.17±7.57                            | 0.004*  | 11.41±5.94                           | 15.17±6.45                           | 0.012   | 16.45±6.07                           | 3.57±1.13                            | 0.009*  |
| Δ Δ CAL (mm) (deepest)   | 3.45±1.87                            | 2.77±1.68                            | 0.017*  | 4.82±1.23                           | 7.04±1.62                           | 0.000*  | 6.73±1.57                           | 7.95±1.60                           | 0.010*  |
| Δ Δ CAL (mm) (deepest)   | 1.73±1.13                            | 1.26±1.76                            | 0.514   | 4.69±2.05                            | 6.58±1.90                            | 0.001*  | 6.73±1.83                           | 8.37±1.86                           | 0.002*  |
| Δ Δ BL (mm) (deepest)    | 1.35±1.07                            | 1.89±1.49                            | 0.163   | 2.37±1.35                            | 3.45±1.87                            | 0.052   | 3.32±1.30                            | 4.61±1.59                            | 0.010*  |

Notes. Data is displayed as mean±SD, *P value ≤0.05 indicates significance.

VI. CONCLUSION

2% chlorhexidine gel used as an intracanal medicament is effective along with periodontal treatment in reducing periodontal inflammation and improving periodontal healing in concomitant endodontic-periodontal lesions with communication as compared to conventional endodontic and periodontal treatment.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES

[1] Bhaskar SN, Orban BJ Orban's oral histology and embryology. St. Louis: Mosby year book. 1991
[2] Abbott PV, Salgado JC. Strategies for the endodontic management of concurrent endodontic and periodontal diseases. Aust Dent J. 2009; 54(suppl. 1): 70-85.
[3] Kobayashi T, Hayashi A, Yoshikawa R, Okuda K, Hara K. The microbial flora from root canals and periodontal pockets of non-vital teeth associated with advanced periodontitis. Int Endod J. 1990; 23: 100-106.
[4] Kurihara H, Kobayashi Y, Francisco IA, Isoshima O, Nagai A, Maruyama Y. A microbiological and immunological study of endodontic-periodontic lesions. J Endod. 1995; 21: 617-621.
[5] Oliver CM, Abbott PV. Correlation between clinical success and apical dye penetration. Int Endod J. 2001; 34: 637-644.
[6] Silva MR, Chambone L, Bombana AC, Lima LA. Early antimicrobial activity of intracanal medications on the external root surface of periodically compromised teeth. Quintessence Int. 2010; 41: 427-431.
[7] Raheja J, Tewari S, Tewari S, Duhon J. Evaluation of efficacy of chlorhexidine intracanal medication on the periodontal healing of concomitant endodontic-periodontal lesions without communication: an interventional study. J Periodontol. 2014; 85: 1019-1026.
[8] Armitage GC. Development of classification system for periodontal diseases and conditions. Ann periodontal. 1999; 4: 1-6.
[9] Abbott PV. Medicaments: aids to success in endodontics. Part I. A review of the literature. Aust Dent J. 1990; 35: 438-448.
[10] Silness I, Loe H. Periodontal disease in pregnancy. I. Correlation between oral hygiene and periodontal condition. Acta Odontologica Scandinavica. 1964; 22: 121-135
[11] Loe H, Silness J. Periodontal disease in pregnancy. I. prevalence and severity. Acta Odontologica Scandinavica. 1963; 21: 533-551.
[12] Trostan L, Barnett F, Riso K, Slots J. Extracellular endodontic infections. Endod Dent Traumatol. 1987; 3: 86-90.
[13] Trostan L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol. 1990; 6: 73-77.
[14] Gomes BP, Montagner F, Berber VB et al. Antimicrobial action of intracanal medicaments on the external root surface. J Dent. 2009; 37: 76-81.
[15] Hugo WB, Longworth AR. Some aspects of the mode of action of chlorhexidine J Pharm Pharmacol. 1964; 16: 655-662.
[16] Kontakiotis EG, Tsatsoulis IN, Papanakou SI. Effect of 2% Chlorhexidine Gel Mixed with Calcium Hydroxide as an Intracanal Medication on Sealing Ability of Permanent Root Canal Filling: A 6-month Follow-up. J Endod. 2008; 34: 866-871.
[17] Grenier D. Effect of chlorhexidine on the adherence properties of Porphyromonas gingivalis. J Clin Periodontol. 1996; 23: 140-142.
[18] Socransky SS, Haftjace AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998; 25: 134-144.
[19] Chaves ES, Jeffcoat MK, Ryerson CC, Snyder B. Persistent bacterial colonization of Porphyromonas gingivalis, Prevotella intermedia, and Actinobacillus actinomycetemcomitans in periodontitis and its association with alveolar bone loss after 6 months of therapy. *J Clin Periodontol* 2000; 27: 897-903.

[20] Gomes BP, Souza SF, Ferraz CCR, Teixeira FB, Zaia AA, Valdrighi L, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. *Int Endod J.* 2003; 36: 267-275.

[21] Rosenthal S, Spangberg L, Safavi K. Chlorhexidine substantivity in root canal dentin. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol and Endod.* 2004; 98: 488-492.

[22] Khademi AA, Mohammadi Z, Havaee A. Evaluation of the antibacterial substantivity of several intra-canal agents. *Aust Endod J.* 2006; 32: 112–115.

[23] Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza Filho FJ. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol Endod.* 2004; 97: 79-84.

[24] Gomes BP, Vianna ME, Zaia AA, Almeida JF, Souza-Filho FJ, Ferraz CC. Chlorhexidine in Endodontics. *Brazilian Dent J.* 2013; 24: 89-102.

[25] Shiloah J, Patters MR. Repopulation of periodontal pockets by microbial pathogens in the absence of supportive therapy. *J Periodontol.* 1996; 67: 130-139.

[26] Camargo SC, Gavini G, Aun CE, Waterfield D, Coil JM. Diffusion of calcitonin through the wall of the root canal. *Braz Oral Res.* 2004; 18: 59-62.

[27] Berber VB, Gomes BP, Sena NT, Vianna ME, Ferraz CCR, Zaia AA, J. et al. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. *Int Endod J.* 2006; 39: 10-17.