Comparative evaluation of two subgingival irrigating solutions in the management of periodontal disease: A clinicomicrobial study

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Abstract:
Context: Local administration of antimicrobial agents offer a “site-specific” approach to the periodontal therapy and it has several benefits. Aim: The present study was aimed to assess the clinical and microbial changes by subgingival irrigation using different subgingival irrigants in periodontitis patients and also to assess the mechanical effect of different local irrigation devices; if any. Settings and Design: Split-mouth design was employed on ten individuals. Materials and Methods: The study sample consisted of 10 individuals in whom full-mouth scaling and root planing was performed and subgingival irrigation therapy was instituted for an experimental period of 30 days. The clinical as well as microbiological parameters were evaluated. Statistical Analysis Used: To calculate baseline data with day thirty data, paired t-test was used. Intergroup comparison was carried out using post hoc Tamhane’s T2 test. Results: Among the different subgingival irrigants used, 0.2% chlorhexidine gluconate is most effective followed by ozonated water, whereas saline was found to be ineffective when compared to the other two subgingival irrigants. Subgingival irrigation using pulsated device may not have any additive effect in alteration of the subgingival microflora. Conclusion: Within the limits and scope of the study, it can be safely concluded that 0.2% chlorhexidine may be used as an adjunct to mechanical therapy for achieving a significant reduction in inflammatory periodontal changes and also reduction in periodontopathogenic microflora.

Key words: Chlorhexidine, ozone, periodontitis, subgingival irrigation

INTRODUCTION

Gingivitis and periodontitis are infectious diseases of bacterial origin. Removal of supragingival plaque is usually sufficient to prevent inflammation. However, effective treatment of periodontitis requires the control of subgingival plaque also. As the pockets deepen, plaque control measures become less effective. Retention of plaque in inaccessible sites can be a nidus for reinfection, which may allow return of pretreatment microflora with recurrence of disease. Systemic and topical chemotherapeutic agents have been used as adjunctive methods for the treatment of periodontitis. Long-term use of systemic antibiotics is contraindicated because of adverse effects and the possible development of bacterial resistance. Topical agents used as mouthrinses are of limited usefulness as they do not appear to penetrate pockets that are deeper than 3 mm and may have poor substantivity. Local administration of antimicrobial agents offers a “site-specific” approach to the periodontal therapy having several benefits; primarily, it is localized to infected sites at high concentrations avoiding the potential adverse reactions inherent to the systemic use of these medications.[1]

Subgingival irrigation may be done by different agents such as water, saline, and antiseptics/antimicrobial agents. These irrigants can be delivered to the site with the commercially available subgingival irrigation systems. These systems developed to deliver the antiseptic/antimicrobial agents deep into the periodontal pocket. Chlorhexidine gluconate 0.2% is shown to have a bactericidal action in subgingival flora when used as an irrigant. As this agents show...
certain side effects such as mucosal desquamation, impaired wound healing, fibroblast attachment to root surface, tooth staining, and altered taste sensation; an alternative agent discussed in dentistry is the use of ozone for subgingival irrigation.

Hence, a study was designed to compare the efficiency of ozonated water with the 0.2% “gold standard” chlorhexidine gluconate in treatment of periodontitis.

MATERIALS AND METHODS

This Clinicomicrobiological study consisted of fifty patients of either sex in the age group of 20–65 years with severe periodontitis. The patients who did not turn up for the follow-up and the patients whose plaque index was not “fair” (Silness and Loe plaque index) were excluded from the study. Finally, only ten patients who fulfilled the criteria were considered for the study. The others were considered as dropouts.

Inclusion criteria
1. Generalized pocket depth of 5–8 mm in all quadrants
2. Patients with no periodontal therapy in the last 1 year
3. No history of antibiotics or of oral antiseptics during the last 6 months
4. Ability to attend the hospital at frequent intervals
5. Patients with similar oral hygiene (according to Silness and Loe plaque index).

Clinical trial design
The nature and design of the clinical trial were explained to the patients in the local language, and written consent was obtained for their participation. Oral hygiene instruction for supragingival plaque control was given. Individuals were asked to brush twice daily using a soft toothbrush and paste according to Bass method.[10] A split-mouth design was employed. Among the samples, the treatments included was fullmouth scaling and root planing along with subgingival irrigation using various irrigants and then they were divided into four groups (Table 1).

Sites undergoing irrigation were isolated with cotton rolls. The subgingival irrigation tip was placed approximately 1–2 mm into the selected pocket to ensure proper placement. The tip was held adjacent to the tooth surface at an angle of approximately 45°.[13] Each pocket was irrigated with irrigating solution using a subgingival irrigating tip for 20 s.[14] Continuous aspiration was used to remove any irrigant that might flow from the pocket orifice to any other area during the irrigation procedure. Emphasis was placed upon carrying out the irrigation with minimal trauma and discomfort. Subgingival irrigation was carried on 1st, 2nd, 3rd, and 4th week by all three irrigants.

Group I
0.2% chlorhexidine gluconate solution was placed in the reservoir of the Water Pik Irrigator device. The Water Pik Irrigator was set at 6 (on a pressure scale from 1 to 10) providing an impact of rigid surface of 0.595 at 350 kpa pressure.

Group II
Subgingival irrigation was done using ozonated water which was obtained from Kent Dental Jet Irrigator where a 27 -gauge blunt needle was first bent and then attached to the tip of the nozzle. The Kent Dental Jet Irrigator was set at 4 (on a pressure scale from 1 to 4) providing an impact of rigid surface of 0.595 at 350 kpa pressure.

Group III
Subgingival irrigation was performed using 0.9N saline in Water Pik Irrigator device. The Water Pik Irrigator was set at 6 (on a pressure scale from 1 to 10) providing an impact of rigid surface of 0.595 at 350 kpa pressure.

Group IV
Only scaling and root planing was performed.

Clinical analysis
Clinical parameters were evaluated using Gingival Index (Loe and Silness 1963) and probing pocket depth on the day 0 and 30 after the treatment.

Microbiological analysis
Plaque samples were taken for microbiological analysis from the mesial aspect of 1st molar from each quadrant within 20 s after the prescribed treatment was completed.[15] Samples were taken on day 0 which was considered as baseline control value, the 15th and 30th day colony counting values were recorded to be assessed against the control group as dependent parameters. The microbiota which was taken into consideration for the present study was Fusobacterium and Bacteroides.

To obtain the plaque sample, paper points were inserted to the depth of the pocket. The points were left undisturbed for 10 s and then transferred immediately to a sterile container containing Robertson’s cooked meat transport media.[16] This was then transported immediately to the laboratory for microbiological analysis.

The sample was mixed with 20 ml liquid broth and vortex mixed. 20 μl of suspension was cultured in selective (neomycin) blood agar and incubated anaerobically using Gas Pak System.

RESULTS

In the clinical analysis, parameters evaluated were Gingival index (Loe and Silness 1963) and probing pocket depth on baseline and 30th day. Microbiological analysis was performed for evaluation of species Fusobacterium and Bacteroides. The values obtained by microbial culture and colony count were divided into 3 categories for each of the four major experimental groups. Categories were baseline, 15 day colony forming unit as a second category, and 30 day colony forming unit was the third category for each of species.

Table 1: Distribution of sample

| Groups   | Quadrant      | Irrigant system used                        |
|----------|---------------|---------------------------------------------|
| Group I  | 1<sup>st</sup> quadrant | Scaling and root planing along with 0.2% chlorhexidine irrigation |
| Group II | 2<sup>nd</sup> quadrant | Scaling and root planing along with ozonated water irrigation |
| Group III| 3<sup>rd</sup> quadrant | Scaling and root planing along with saline irrigation |
| Group IV | 4<sup>th</sup> quadrant | Scaling and root planing alone |

598
The baseline values in Group IV were considered as control, and these values were assessed against the experimental values in Group I, II, and III by paired sample \(t\)-test for significance in difference of means to reject the null hypothesis that the experimental groups do not have any significant difference than baseline. A paired sample \(t\)-test was also used in a similar way to test the significance between baseline values of probing pocket depth and gingival index. The intergroup comparison was done using one-way ANOVA test and for multiple comparison where equal variance was not assumed, Tamhane’s \(T_2\) test was used.

**Probing pocket depth**

Comparison of probing pocket depth at baseline and day 30 was done using paired \(t\)-test. Group I showed a mean difference of 1.87600 (standard deviation [SD] of ± 0.56038) which was seen to be significant \((P = 0.000)\). Similarly, Group II, III, and IV also showed mean difference of 1.46100, 0.81200, and 0.61900, respectively, with a SD of ± 0.63278, ± 0.67199, and ± 0.31918 \((P = 0.000, P = 0.004, P = 0.000\) respectively) which were all significant [Table 2a].

The intergroup comparison for the probing pocket depth was done by ANOVA on baseline and on day 30 [Table 2b]. To compare baseline data with day 30 data between control group (Group IV) and experimental groups (Group I, II, III), Tamhane’s post hoc test was used. The day 30 intercomparison between Group IV to Group I showed mean difference of a 1.24500 which was significant \((P = 0.002)\); Group IV and Group II showed mean difference of a 1.02800 which was significant \((P = 0.043)\) but comprising Group IV and Group III showed mean difference of a 0.37200 which was nonsignificant \((P = 0.732)\) [Table 2c].

**Gingival index**

Comparison of gingival index at baseline and day 30 was done using paired \(t\)-test [Table 3a]. The day 30 intercomparison showed a mean square value of 2.242 with \(F = 17.449\) which was significant \((P = 0.000)\) [Table 3b].

The intergroup comparison was done using one-way ANOVA test and for multiple comparison where equal variance was not assumed, Tamhane’s \(T_2\) test was used. The day 30 intercomparison between Group IV to Group I showed mean difference of 1.00500 which was significant \((P = 0.001)\); Group IV and Group II showed mean difference of a 0.65500 which was significant \((P = 0.021)\) but comparison of Group IV and Group III showed mean difference of a 0.10300 which was nonsignificant \((P = 0.996)\) [Table 3c].

**Fusobacterium**

Paired sample \(t\)-test was performed to analyze *Fusobacterium* count between baseline to day 15 and baseline to day 30 [Table 4a].

The intergroup experimental value comparison of mean for the *Fusobacterium* count was done by one-way ANOVA on baseline, day 15, and day 30. The day 30 values between groups showed mean square value of 5.313 with \(F\) value of 7.329 which was significant \((P = 0.001)\) [Table 4b].
The Tamhane’s post hoc multiple comparison test was applied for comparison between groups, where control Group IV values were compared with the other three experimental groups for baseline, day 15, and day 30 [Table 4c].

**Bacteroides**

Paired sample t-test was performed to analyze Bacteroides count between baseline to day 15 and baseline to day 30 [Table 5a].

The intergroup experimental value comparison of mean for the Bacteroides count was done by one way ANOVA on baseline, day 15, and day 30. On baseline, the mean square value observed was 2,291,666.667 with $F = 0.3440$ which was found to be nonsignificant ($P = 0.794$). Day 15 value showed a similar pattern in which mean square value was 002.627 with $F$ value of 5.447 which was significant ($P = 0.003$). The day 30 values between groups showed mean square value of 004.609 with $F$ value of 17.522 which was also significant ($P = 0.000$) [Table 5b].

In Tamhane’s post hoc multiple comparison test, Group IV values were compared with the other three experimental groups for baseline, day 15, and day 30. While comparing between control group IV with experimental Groups I, II and III on day 0, mean difference of 400,000, -400,000 and 700,000 were found respectively, which was statistically nonsignificant ($P > 0.05$). Day 15 values also showed the same trend. The 30th day count values when compared for Group I and II with control Group IV showed a mean difference of 520,000 and 2700,000, respectively, which were statistically significant ($P = 0.000, P = 0.040$) [Table 5c].

**DISCUSSION**

Previous investigators have performed daily or biweekly subgingival irrigation with different irrigants. We chose three different professionally applied irrigations at intervals of 1 week to simulate clinical practitioners who often schedule scaling and root planing quadrant wise weekly.[7]

Fusobacterium was taken as an indicator for destructive periodontal status in the present study based upon the studies by Sigusch et al.[1] in which periodontal immune mechanism is found to be impaired because of neutrophil disfunction by Fusobacterium. In another study by Sheikh et al.[8] the possible contribution of Fusobacterium species and polymorphonuclear neutrophils to the disease processes of periodontitis was evaluated.

In another study by Newman et al.[9] an evaluation of the involvement of Fusobacterium nucleatum clinical strains in adult periodontitis by subspecies and expression of hemagglutination activity was assessed. Holt et al.[10] studied the effect of implantitis on Bacteroides gingivalis in nonhuman primates and studied its effect on initiation and progression of periodontitis.

White and Mayrand[11] studied the association of oral Bacteroides with gingivitis and adult periodontitis.

Tran et al., in his research work suggested the persistent presence of Bacteroides forsythus as a risk factor for attachment loss in a population with low prevalence and severity of adult periodontitis. Our present study focused on a similar concept by exploring for a corelation and association if any, between the local irrigation with various chemical agents and microbiological parameters with Bacteroides and Fusobacterium as representative of the microbial periodontopathogenic microflora of experimental site.

There are several ways of delivering chemical agents. One of the ways such as the subgingival irrigation interferes with the complex ecosystem required for the initiation and
Ozonated water has been shown to be effective against periodontopathogenic bacteria such as Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in vitro. Ozonated water has also been shown to be effective on root surfaces after extraoral rinsing for decontamination of avulsed tooth in vitro. Although there are studies related to the use of ozonated water on oral microorganisms in vitro, no literature exists till date on the in vivo effect of ozonated water on oral and in particular, periodontopathogenic microorganisms. Ozone is a selective oxidant and affects only certain compounds, but when it dissolves in water, it becomes highly unstable and rapidly decomposes through a complex series of chain reactions. As a result, hydroxyl (OH) radicals are generated which are among the most reactive oxidizing species. Ozone reacts with various chemical compounds in aqueous systems in two different and coexisting modes; one involving direct reactions of molecular ozone and the other a free radical-mediated reaction. Both these mechanisms may be involved in the destruction of bacteria as shown by Kshitish and Laxman.[14] To assess the mechanical effect of different local irrigational methods, Group III that is saline irrigation group was compared with Group IV. In Group III, irrigation was performed with use of pik pocket tip of water pik irrigator wherein the data showed statistically insignificant results, suggesting that subgingival irrigation using pulsed device does not have any additive effect over mechanical debridement. Data were not consistent with results of Braun et al. who evaluated that subgingival irrigation of pockets 1-6 mm deep with a pulsed powered irrigator using a subgingival irrigating tip is effective in delivering a solution to 90% of pocket depth.

Considering the limitation of this study in terms of short-term duration, ozone along with chlorhexidine which is already proved as a gold standard can be considered as a promising antimicrobial agent in the periodontal therapy.

It is required to determine the specific ozone concentration that is effective against anaerobic periodontopathogens. Because of the purpose of this preliminary trial was to study the effect of ozone on a couple of the periodontopathogens from the active, deep periodontal pockets of periodontitis patients, pooled plaque samples were collected from the selected sites. However, site-specific studies will be more relevant. It can be concluded that the local application of ozone can serve as a potential agent to treat periodontal disease nonsurgically, both, for home care and for professional practice. It may serve as a good tool during supportive periodontal therapy.

**CONCLUSION**

The mechanical therapy with or without subgingival irrigation does lower the bacterial load. It was evident from the reduced counts of *Fusobacterium* and *Bacteroides* that the use of subgingival irrigation therapy had better results. Subgingival irrigation with an antimicrobial agent for a duration of 30 days as an adjunct to mechanical therapy enhances periodontal health and may have a significant role in periodontal therapy.

Among the different subgingival irrigants used, 0.2% chlorhexidine gluconate is most effective followed by ozonated water, whereas, saline was found to be ineffective when compared to the other two subgingival irrigants.

Subgingival irrigation using pulsed device may not have any additive effect in alteration of the subgingival microflora.

Within the limits and scope of the study, it can be safely concluded that 0.2% chlorhexidine may be used as an adjunct to mechanical therapy for achieving a significant reduction in inflammatory periodontal changes and also reduction in periodontopathogenic microflora.

### Table 5b: *Bacteroides*: Intergroup comparison of *Bacteroides* between groups at baseline, day 15, and day 30

| Day    | Intergroup comparison          | Mean square | F     | Significance |
|--------|--------------------------------|-------------|-------|--------------|
| Day 0  | Between groups                 | 2,291,666.667 | 0.3440 | 0.794 (NS)   |
| Day 15 | Between groups                 | 2.627       | 5.4470 | 0.003 (S)    |
| Day 30 | Between groups                 | 4.609       | 17.522 | 0.000 (S)    |

*Mean difference is significant at the 0.05 level. NS – Not significant; S – Significant

### Table 5c: *Bacteroides*: Multiple comparisons at baseline, day 15, and day 30 of *Bacteroides*

| Dependent variable | Control group | Groups | Mean difference | Significance |
|--------------------|---------------|-------|-----------------|--------------|
| Day 0              | 4             | 1     | 0400.000        | 1.000 (NS)   |
|                    | 2             | -0400.000 | 1.000 (NS)    |
|                    | 3             | 0700.000 | 0.878 (NS)     |
| Day 15             | 4             | 1     | 3800.000        | 0.015 (NS)   |
|                    | 2             | 1000.000 | 0.938 (NS)     |
|                    | 3             | 1200.000 | 0.664 (NS)     |
| Day 30             | 4             | 1     | 5200.000        | 0.000 (S)    |
|                    | 2             | 2700.000 | 0.040 (S)      |
|                    | 3             | 2000.000 | 0.112 (NS)     |

*The mean difference is significant at the 0.05 level. NS – Not significant; S – Significant

continued destruction of the compromised periodontium in the susceptible host. The effects of irrigation on gingival bleeding and plaque include change in plaque composition, flushing out of inflammation-inducing factors, and physical change in tissue integrity.[13]

Chlorhexidine has emerged as an important oral antibacterial agent and adjunct to periodontal therapy. It is a broad-spectrum antiseptic with pronounced antimicrobial effects on Gram-positive as well as Gram-negative bacteria, some viruses, and fungi.[14] The combined use of irrigators and chlorhexidine appears to be more effective than when used as a mouthrinse at altering the subgingival microflora. Khoo and Newman noted reductions in motile organisms and spirochetes following daily irrigation with 0.2% chlorhexidine as compared with a single session of scaling, root planing, and oral hygiene instruction.

Oxygen too has been used for subgingival irrigation. A few oxygenating agents such as sodium monohydrate, sodium oxychlorosene, carbamide peroxide, and sodium borate peroxhydroxyde were applied to areas of wounded rat oral tissue. All of these showed complete healing in a shorter time than normally required. Recently, an allotropic form of oxygen, ozone, is being used in dentistry for the treatment of dental caries.[14]

Ozonated water has been shown to be effective against periodontopathogenic bacteria such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in vitro. Ozonated water has also been shown to be effective on root surfaces after extraoral rinsing for decontamination of avulsed tooth in vitro. Although there are studies related to the use of ozonated water on oral microorganisms in vitro, no literature exists till date on the in vivo effect of ozonated water on oral and in particular, periodontopathogenic microorganisms. Ozone is a selective oxidant and affects only certain compounds, but when it dissolves in water, it becomes highly unstable and rapidly decomposes through a complex series of chain reactions. As a result, hydroxyl (OH) radicals are generated which are among the most reactive oxidizing species. Ozone reacts with various chemical compounds in aqueous systems in two different and coexisting modes; one involving direct reactions of molecular ozone and the other a free radical-mediated reaction. Both these mechanisms may be involved in the destruction of bacteria as shown by Kshitish and Laxman.[14] To assess the mechanical effect of different local irrigational methods, Group III that is saline irrigation group was compared with Group IV. In Group III, irrigation was performed with use of pik pocket tip of water pik irrigator wherein the data showed statistically insignificant results, suggesting that subgingival irrigation using pulsed device does not have any additive effect over mechanical debridement. Data were not consistent with results of Braun et al. who evaluated that subgingival irrigation of pockets 1-6 mm deep with a pulsed powered irrigator using a subgingival irrigating tip is effective in delivering a solution to 90% of pocket depth.

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Conflicts of interest
There are no conflicts of interest.

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