INTRODUCTION

Periodontal infection is initiated by specific invasive oral pathogens that colonize dental plaque biofilms on tooth surface, and host immune response to inflammation plays a central role in disease pathogenesis. Scaling and root planning (SRP) is the gold standard approach for treatment of chronic periodontitis but used alone it may not be effective in removing periodontal pathogens from sites where access is poor.

OBJECTIVE: To evaluate and compare the clinical and microbiological efficacy of ozone and chlorhexidine (CHX) as an adjunct to SRP in patients with chronic periodontitis.

METHODS: Twenty-five patients with generalized moderate to severe chronic periodontitis with presence of at least one site in each quadrant with a probing depth ≥5 mm were recruited. In a split mouth study design, two quadrants were randomly allocated to the SRP and ozone therapy and the remaining two quadrants to SRP and CHX therapy. Plaque index (PI), Gingival index (GI), probing depth (PD), clinical attachment loss (CAL) were assessed. Subgingival plaque samples were obtained for assessment of Aggregatibacter actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg).

RESULTS: Both groups demonstrated significant intragroup reduction in PI, GI, PD, CAL, Pg count and Aa count from baseline to 3 months follow-up. There were no significant differences between two groups for any of the parameters.

CONCLUSION: Ozonated olive oil can be used as an adjunctive subgingival irrigant in patients with chronic periodontitis.

Effectiveness of the adjunctive use of ozone and chlorhexidine in patients with chronic periodontitis

Kaveri Kranti Gandhi1, Emil G. Cappetta2 and Rajdeep Pavaska3

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CHX and ozonated olive oil on clinical parameters—PI, GI, PD and CAL. (2) To assess and compare the effect of CHX and ozonated olive oil on Pg and Aa counts by using bacterial culture method. The protocol was approved by the author's institutional review committee for human subjects and the study was conducted in accordance with the Helsinki Declaration as revised in 2013.

**MATERIALS AND METHODS**

**Patient selection and study design**

A total of 25 patients, 30–60 years of age suffering from generalized moderate to severe chronic periodontitis, with the presence of at least one site in each quadrant with a probing depth ≥5 mm were selected. Patients with history of any systemic diseases, smokers, chronic alcoholics, pregnant and lactating women, patients who had received any periodontal therapy in last 6 months, patients with the history of use of any oral rinse or antibiotic therapy in last 6 months were excluded from the study. A randomized, double-blind, split mouth study design was performed. Randomization was carried out using computer generated random numbers. All treatment procedures were performed by a single periodontist. Clinical measurements were recorded and subgingival plaque sampling was carried out in all patients by another examiner. The following clinical parameters were recorded in all patients: PI according to Silness, P. and Loe, H. (1964); GI according to by Loe H and Silness J (1963); PD—It was measured from base of the pocket to the gingival margin at mesiobuccal, midbuccal, distobuccal and lingual surfaces using UNC-15 probe; CAL—It was measured from base of the pocket to the cementoenamel junction at mesiobuccal, midbuccal, distobuccal and lingual surfaces using UNC-15 probe. For microbiological evaluation, pooled subgingival plaque samples were obtained from all sites in each group with PD ≥ 5 mm at baseline. These sites were isolated with cotton rolls, following which supragingival plaque was removed using a sterile hand scaler and cotton gauge, to prevent any contamination of the samples. Subgingival plaque samples were obtained using a sterile Gracey curette and sent for microbiological examination in a sterile container containing RTF (reduced transport fluid).

Informed consent was obtained from each patient after explaining the aims and objectives of the study. Clinical parameters were recorded, and subgingival plaque samples were collected at baseline before SRP and 3 months after the treatment.

**Treatment procedure**

SRP was carried out in all patients using ultrasonic and hand instruments in two visits. In the first appointment, each patient received full mouth supragingival scaling with a piezoelectric handpiece (EMS). After 1 week, on the second appointment, full mouth subgingival SRP was done using both Gracey curettes (Hu-Friedy) and piezoelectric handpiece under local anesthesia of 2% lidocaine with 1:100,000 epinephrine. Following which, two quadrants were assigned to the CHX group and the remaining two quadrants were assigned to the ozonated olive oil group. All sites with probing depth ≥5 mm were isolated carefully with cotton rolls and thoroughly dried and CHX (0.2%) or ozonated olive oil was applied carefully subgingivally with the help of a disposable 2 ml plastic syringe and a 28-gauge needle. Patients were instructed not to eat, drink or rinse for at least 30 min. Subgingival application of ozonated olive oil and chlorhexidine was performed immediately after SRP on the second visit and was repeated 2 weeks after SRP.

Power calculation and statistical analysis

Using G* Power 3.1.9.4 software the sample size was estimated to be 20. A sample size of 25 was recruited to compensate for the possible dropouts during the study. The sample size estimation before the start of the study considered beta error to be 20%.

Post-hoc power analysis done after completion using G* Power 3.1.9.4 software suggested that the power achieved in the present study is 100%. Statistical analysis was performed using the statistical software SPSS version 20. For statistical analysis only sites with PD ≥ 5 mm at baseline were included in the analysis. Intragroup differences in clinical and microbiological parameters were analyzed by paired t test whereas the intergroup differences were analyzed using unpaired t test. Statistical significance was set at 95% probability level (P < 0.05).

**RESULTS**

All patients attended the third month follow-up appointment. No discomfort or side effects were reported by any of the patients. At baseline examination, there were no statistically significant differences between the groups with regard to any of the recorded parameters. Both the groups demonstrated significant intragroup reduction in PI, GI, PD, CAL, Pg count and Aa count from baseline to 3 months follow-up. However, on intergroup comparison, no statistically significant differences were found between the CHX and ozonated olive oil groups regarding any of the clinical and microbiological parameters at the follow-up visit (Tables 1–6).

**DISCUSSION**

The mechanical removal of plaque and calculus and the adjunctive use of antibiotics and antiseptics have been the conventional methods for periodontal therapy. The powerful antimicrobial action of ozone, with its ability to modulate the conventional methods for periodontal therapy. The powerful antimicrobial action of ozone, with its ability to modulate the...
Table 4. Comparison of mean values of clinical attachment loss

| Groups             | Baseline | At 3 mo. | Difference | P value |
|--------------------|----------|----------|------------|---------|
| Ozonated olive oil | 5.02 ± 0.37 | 2.92 ± 0.43 | 2.10 ± 0.27 | 0.00^a |
| Chlorhexidine      | 4.78 ± 0.37 | 2.63 ± 0.40 | 2.12 ± 0.48 | 0.00^a |
| Difference         | 0.06     | 0.23     |            |         |
| P                  | 0.96     | 0.68     |            |         |

*Represents statistically significant difference

Table 5. Comparison of mean values of bacterial counts of Porphyromonas gingivalis

| Irrigants         | Baseline | At 3 mo. | Difference | P value |
|-------------------|----------|----------|------------|---------|
| Ozonated olive oil| 77.80 ± 38.52 | 0.90 ± 0.87 | 76.90 ± 38.09 | 0.00^a |
| Chlorhexidine     | 82 ± 33.35 | 0.90 ± 0.87 | 81.10 ± 32.85 | 0.00^a |
| Difference        | 4.20     | 0.00     |            |         |
| P                 | 0.75     | 1.00     |            |         |

*Represents statistically significant difference

Table 6. Comparison of mean values of bacterial counts of Aggregatibacter Actinomycetemcomitans

| Groups            | Baseline | At 3 mo. | Difference | P value |
|-------------------|----------|----------|------------|---------|
| Ozonated olive oil| 19.00 ± 15.59 | 0.20 ± 0.42 | 18.80 ± 15.33 | 0.00^a |
| Chlorhexidine     | 22.00 ± 12.97 | 0.30 ± 0.67 | 21.70 ± 12.63 | 0.00^a |
| Difference        | 3.00     | 0.10     |            |         |
| P                 | 0.55     | 0.33     |            |         |

*Represents statistically significant difference

ozone is known to activate angiogenesis. This process is brought about by the secretion of vasodilators like nitric oxide (NO). Nitric oxide causes vasodilation of arterioles and releases growth factors like vascular endothelial growth factor (VEGF) which helps in angiogenesis. Topical administration of ozone can be performed in gaseous form through an open system or through a suction or as ozonated water and ozonated oil. In this study, ozonated olive oil was selected over ozonated water because the application of oil has been found to provide a long stay in the oral cavity, adequate drug penetration, high efficacy and acceptability. A split-mouth design was used to eliminate patient-specific conditions and allow the comparison of both treatment methods under similar conditions.

The use of ozone therapy in treatment of chronic periodontitis has been evaluated by many studies. While some studies showed the additional benefit of using ozone therapy, others demonstrated that the adjunctive use of ozone therapy provided no additional benefit.

In our study, no significant difference was found in the efficacy of ozonated olive oil and CHX in improving the PI, GI, PD, clinical attachment levels and reducing the PD and Aa. However, our results differ from those obtained by Kshitish and Laxman. They observed a higher percentage of reduction in PI, GI, and bleeding index and Aa using ozone as compared to using CHX. Another study compared the effectiveness of ozone with that of the CHX, against periodontal microorganisms reported no significant differences in the effectiveness of aqueous ozone or gaseous ozone compared with 2% CHX but they were more effective than 0.2% CHX.

Ramzy et al. reported a highly significant improvement in clinical parameters and bacterial count in quadrants treated by SRP together with ozone application in comparison to SRP alone in patients with aggressive periodontitis. On the other hand, irrigation with ozonated water as an adjunctive therapy to SRP produced no statistically significant benefit compared with SRP plus distilled water irrigation in the study by Al Habashneh et al. However, both these studies did not test the efficacy of CHX in comparison to ozone. Müller et al. compared the influence of ozone gas with photodynamic therapy and antibiotic agents to 2% CHX, 0.5% hypochlorite solutions on a multispecies oral biofilm in vitro. Actinomycyes naeslundii, Veillonelladispar, Fusobacterium nucleatum, Streptococcus sobrinus, Streptococcus oralis and C. albicans were studied. They concluded that the matrix-embedded microbial populations in biofilm are well protected towards antimicrobial agents. Only 5% hypochlorite solution was able to eliminate all bacteria effectively. Usage of gasform ozone or PDT was not able to reduce bacteria in the biofilm. In a study that evaluated the antimicrobial efficacy of Er:YAG laser and topical gaseous ozone, the authors found that ozone has antimicrobial effect equivalent to that of the Er:YAG laser.

CONCLUSION

The adjunctive use of ozonated olive oil in the treatment of chronic periodontitis significantly improves clinical and microbiological results and is equally effective as chlorhexidine, and at the same time is free from adverse effects.

ADDITIONAL INFORMATION

Competing interest: The authors declare no competing interest.

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