ORIGINAL RESEARCH REPORT

Efficacy of ozonated water and chlorhexidine mouth rinse against plaque and gingivitis: A randomized clinical trial

Sujal Mitul Parkar, Kanchi Shah[1], Nikhil Darjee[1], Abhishek Sharma[2]

Department of Public Health Dentistry, Siddhpur Dental College and Hospital, Siddhpur, [1]Department of Public Health Dentistry, Ahmedabad Dental College and Hospital, Ahmedabad, Gujarat, [2]Department of Public Health Dentistry, Government Dental College and Hospital, Jaipur, Rajasthan, India

Address for correspondence:
Dr. Sujal Mitul Parkar,
B-25 Krishna Bunglows-I,
Gandhinagar Highway, Motera,
Ahmedabad - 380 005,
Gujarat, India.
E-mail: drsujal_pcd@live.com

ABSTRACT

Aim: The aim of this study was to compare the efficacy of ozonated water and chlorhexidine mouth rinse against plaque and gingivitis. **Materials and Methods:** A randomized, double-blind clinical trial was conducted. A total of 54 patients suffering from generalized chronic gingivitis were chosen for the study. The trial period of 15 days was divided into three time-intervals, that is, baseline (0 day), 7th day and 15th day. The use of ozonated water, 0.2% chlorhexidine, and water irrigation was randomized. The clinical parameters such as plaque and gingival status were assessed using Turkesky-Gilmore-Glickman Modification of Quigley – Hein Plaque Index and Loe and Silness Gingival Index, respectively. The repeated one-way analysis of variance followed by *post hoc* Bonferroni test was used for statistical analysis. The level of significance was considered at 5%. **Results:** The analysis was performed on 47 patients at the end of 15th day. There was a highly significant difference ($P < 0.001$) when the mean plaque and gingival scores were compared between three different interval of time. There was a significant difference ($P = 0.002$) when the mean plaque scores for chlorhexidine and water were compared. There was a significant difference when the mean gingival scores for ozonated water and chlorhexidine ($P = 0.002$) were compared. **Conclusion:** The results of this study demonstrate that ozonated water was as equally effective as chlorhexidine in reducing plaque and gingivitis with slight edge over chlorhexidine.

Key words: Chlorhexidine, mouthwash, ozone, randomized clinical trial

INTRODUCTION

Dental plaque is a living, organized community of microorganisms, consisting of numerous species embedded in an extracellular matrix, known to be the main etiological factor for gingival and periodontal diseases. The current treatment for plaque induced gingivitis is directed at disruption of plaque maturation and/or reduction of bacterial load on tooth surface, which can be effectively achieved through plaque control. Plaque control is a critical component of dental practice, permitting long-term success of periodontal and dental care. There is a growing interest throughout the oral health care profession in therapeutic agents that compliments and enhances the mechanical removal of biofilms in the oral cavity.[1]

Despite the potential for adequate mechanical plaque control clinical experience, the population-based studies demonstrate that such methods are not being employed sufficiently by large number of the population[2,3] as it is highly labor intensive whether professionally administered or practiced personally. Not surprisingly, therefore large numbers of chemical agents in the form of

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mouthwashes have been tested for their ability to reduce plaque accumulation in patients who cannot maintain adequate mechanical plaque control. They can, therefore, be considered a less technically demanding adjuvant to mechanical control.[4]

Many chemical adjuncts have been tried along with mechanical oral hygiene procedure for plaque control out of which, chlorhexidine gluconate (0.2%) was found to be very effective. Chlorhexidine is a cationic bisbiguanide which is effective against array of microorganisms, including Gram-positive and Gram-negative organisms, fungi, yeast and viruses. Some common side effects of chlorhexidine reported till date are desquamation of oral mucosa, delayed wound healing, tooth staining, and altered taste sensation.

Ozone is currently being discussed in dentistry as a possible alternative antiseptic agent. Recent investigations have reported that the gaseous as well as aqueous forms of ozone are equally effective as anti-microbial agents against oral pathogens,[5-7] and the effectiveness of ozone in the treatment of oral diseases is currently a subject of intensive research.[8] To consider ozone as a potential antimicrobial agent against oral pathogens, it is important to compare its effectiveness with established agents currently used in dentistry.

Through extensive literature search, it was revealed that there is a lack of researches conducted on the effects of ozonated water on clinical parameters of dental plaque and gingivitis. Hence, the aim of the present study was to evaluate and compare the effect of oral irrigation with ozonated water and 0.2% chlorhexidine on the plaque and gingival scores among the patients suffering from chronic generalized gingivitis.

MATERIALS AND METHODS

To determine the appropriate sample size a pilot study was conducted. The result of pilot study showed the mean difference for gingival score of 0.35. Considering the mean difference of 0.35, 80% power $(1 - \beta)$ of study and 95% confidence interval $(1 - \alpha/2)$ the sample size was calculated as 54.

Fifty-four individual age ranges from 18 to 30 years attending the Department of Public Health Dentistry were enrolled in this double-blind, parallel, controlled clinical trial. All the participants were informed about the nature of the study. The informed consent was obtained from those who were willing to participate and the ethical clearance was obtained from the Institutional Ethics Committee of Ahmedabad Dental College and Hospital (ADCH).

Inclusion criteria

1. All subjects had normal occlusion with at least 28 teeth present
2. All subjects had the absence of caries and/or any restorations
3. Subject suffering from chronic generalized gingivitis
4. None of the subjects were wearing fixed or removable orthodontic appliances or partial dentures
5. Subject agreement to use only oral care products provided by the investigator during the study.

Exclusion criteria

1. History of any systemic illness such as hypertension, liver disease, epilepsy, convulsion
2. Allergy or hyper sensitivity to any chemical component in the oral care products used for the study
3. Use of antibiotics or any other medications taken within 7 days of the baseline
4. Subjects having periodontal tissue breakdown or any pathological lesion.

Clinical design

All 54 subjects were randomly assigned into three groups: 18 subjects in each group. Group 1: Ozonated water (Ozone Purifier, Eltech Engineers, Mumbai, India), Group 2: 0.2% Chlorhexidine mouthwash (Hexidine®, ICPA, Mumbai, India), and Group 3: Water (Placebo group). The random allocation of the study subjects in each group was performed by using lottery method.

Clinical examination

The clinical examination was carried out by trained examiners (SK and DN) under the direction of (PSM) in the Department of Public Health Dentistry, ADCH. There was a high degree of conformity between two investigators ($\kappa = 0.85$) when Kappa statistics was applied. The clinical parameters like plaque and gingival status was assessed using Turkesky-Gilmore-Glickman Modification of Quigley Hein (1970) Plaque Index[9] and Loe and Silness (1963) Gingival Index,[10] respectively.

The subjects were examined for their plaque and gingival status at baseline (0 day). To blind the investigators and the subject, the participant were randomly allocated one of the three experimental products filled in coded identical bottles using lottery method. For the next 15 days of the study, the subjects were instructed to rinse their mouth with 10 ml of mouth wash two times a day (after breakfast and after dinner) for 1 min. The subjects were told to refrain from all other oral hygiene measures such as dental floss, chewing gum except the directed ones and routine tooth brushing habits and to avoid eating or drinking for at least 30 min after rinsing the mouth. To monitor compliance and record the daily use of the rinse, a dairy was provided and subjects were asked to keep record of the number of bottles used. The subjects were contacted by telephone at different intervals and asked to report bottle number and level of liquid remaining as assessed to each bottle. All bottles were returned at the completion

of the study. The subjects were recalled twice during the experiment to record the clinical parameters precisely after 7 days and after 15 days.

Statistical analysis

Statistical Package for Social Science version 17, (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. The distribution of data was analyzed using Kolmogorov–Smirnov test. Repeated measure analysis of variance (ANOVA), general linear model with partial eta-squared statistics and a α – level of 0.05 was used to test the effects of agents at different time during the trial. The multiple comparisons among the groups were carried out using *post hoc* Bonferroni test. The level of significance was set at 5%.

RESULTS

Fifty-four subjects were enrolled in the study, 18 subjects in each group. Four subjects from Group 2 and 3 subjects from Group 3 did not turn up for follow up and were not able to complete the trial of 15 days hence, 47 subjects out of 54 remained at the end of the trial with the attrition rate of 17%. The analysis of 47 subjects was carried out at the end of 15th day. There were total 22 male and 25 female subjects with the mean age of 20.98 ± 2.76 years.

A repeated measure ANOVA with Greenhouse-Geisser correction determined that mean values of plaque [Table 1] and gingival score [Table 2] showed a statistically significant difference ($P < 0.001$) between three different interval of time. Bonferroni *post hoc* test was used for multiple comparisons among mean plaque scores [Table 3] and gingival scores [Table 4] for three groups. Table 3 shows the mean difference for plaque scores (−0.33) between chlorhexidine and water was statistically significant ($P = 0.002$). The mean difference (0.25) for gingival scores was significant ($P = 0.002$) when ozonated water and chlorhexidine was compared. However, highly significant difference ($P < 0.001$) observed when the mean difference scores for ozonated water versus water (−0.28) and chlorhexidine versus water (−0.53) were compared [Table 4].

Table 1: Comparison of mean±standard deviation plaque scores at different time intervals

Groups	Baseline (o day)	7 days	15 days	Δ1	Δ2	Δ3	*P*
Ozonated water (*n*=18)	2.78±0.42	2.12±0.26	1.67±0.29	0.66	0.45	1.11	<0.001**
Chlorhexidine (*n*=14)	2.47±0.31	2±0.27	1.53±0.17	0.47	0.47	0.94	
Water (*n*=15)	2.35±0.36	2.29±0.21	2.33±0.31	0.06	−0.04	0.02	
Total	2.55±0.41	2.14±0.27	1.84±0.43				

Δ1=Mean difference baseline (o day) to 7 days, Δ2=Mean difference 7-15 days, Δ3=Mean difference baseline (o day) to 15 days, **Highly significant *P*<0.001

Table 2: Comparison of mean±standard deviation gingival scores at different time intervals

Groups	Baseline (o day)	7 days	15 days	Δ1	Δ2	Δ3	*P*
Ozonated water (*n*=18)	1.92±0.32	1.26±0.25	0.66±0.25	0.66	0.60	1.26	<0.001**
Chlorhexidine (*n*=14)	1.58±0.29	1.05±0.17	0.46±0.14	0.53	0.59	1.12	
Water (*n*=15)	1.63±0.32	1.51±0.21	1.56±0.29	0.12	−0.05	0.07	
Total	1.72±0.34	1.27±0.27	0.89±0.53				

Δ1=Mean difference baseline (o day) to 7 days, Δ2=Mean difference 7-15 days, Δ3=Mean difference baseline (o day) to 15 days, **Highly significant *P*<0.001

Table 3: Multiple comparisons for mean plaque scores between groups

Groups	Mean difference	*P*	95% CI
Ozonated water versus chlorhexidine	0.19	0.08	−0.02-0.41
Ozonated water versus water	−0.13	0.35	−0.35-0.75
Chlorhexidine versus water	−0.33	0.002*	−0.55-−0.11

*Significant *P*<0.05. CI=Confidence interval

Table 4: Multiple comparisons for mean gingival scores between groups

Agents	Mean difference	*P*	95% CI
Ozonated water versus chlorhexidine	0.25	0.002*	0.07-0.42
Ozonated water versus water	−0.28	<0.001**	−0.45-−0.12
Chlorhexidine versus water	−0.53	<0.001**	−0.71-−0.35

*Significant *P*<0.05, **Highly significant *P*<0.001. CI=Confidence interval

DISCUSSION

The initiation and progression of periodontal disease is characterized by the bacterial accumulations on the tooth surface both supra-gingivally and sub-gingivally. Various researches over the period of time have confirmed that effective removal of dental plaque is essential to dental and periodontal health throughout life. Hence, the success of periodontal therapy is to eliminate the sub-gingival bacterial load and also by supra-gingival plaque control measures so that it prevents or minimizes re-colonization of the sub-gingival microflora. Mechanical plaque removal is the most common method used along with non-surgical periodontal therapy. However, the effectiveness of mechanical plaque control is limited by such factors as individual motivation, inaccessibility to periodontal pockets, inter proximal areas, and the margins of restorations. Alternatively, chemical plaque agents has to be used in adjunct to mechanical plaque control.[11]

Chlorhexidine-based formulas are considered as the gold standard for antimicrobial mouthwashes. Chlorhexidine gluconate available in liquid form is a cationic biguanide with broad-spectrum antimicrobial action. Its mechanism of action is that the positively charged chlorhexidine binds to the negatively-charged bacterial cell walls. At

lower concentration this results in increased permeability with leakage of intracellular components. At higher concentration it causes precipitation of bacterial cytoplasm and cell death. Secondary interactions causing inhibition of proteolytic and glycosidic enzymes may also be significant.[12]

Ozone gas become highly unstable and reactive when it comes in contact with water, due to this a complex series of chain reactions occur generating a highly reactive hydroxyl (OH^-) radicals.[13,14] The destruction of bacterial cell is due to the increase in the membrane permeability because of oxidation process. The oxidation process involves two mechanisms; one is direct reaction of ozone molecule in the aqueous system and other is the reaction due to its free hydroxyl (OH^-) radical.[15] Both these mechanisms may be involved in the destruction of bacteria by ozone.

The primary aim of this trial was to evaluate the efficacy of ozonated water on plaque and gingivitis during the short period of 15 days. It has been reported that the periodontal pathogens may survive after nonsurgical periodontal interventions between 1st week to and 1 month[11,16] hence, this study was conducted for short duration of time.

The subjects were asked to rinse with the ozonated water. The rinsing effect of ozonated water interferes with the complex ecosystem required for the initiation and continued destruction of the compromised periodontium in the susceptible host. Various advantages of ozone in the aqueous phase are its potency, ease of handling, lack of mutagenicity, rapid microbicidal effects, and suitability for use as a soaking solution for medical and dental instruments.[17] However, some of the side effects as reported by Matsumura *et al*.[18] are epiphora, upper respiratory irritation, difficulty in breathing, cough, headache, occasional nausea, vomiting, poor circulation, heart problems and at a times stroke.

In the present study, there was a highly significant difference when the mean plaque and gingival score were compared. As in previous reports,[12,19] rinsing twice a day with 0.2% chlorhexidine produced a significant difference in plaque and gingivitis levels as compared to water (placebo) rinse. The results of the present study shows that twice rinsing with ozonated water still reduces gingivitis significantly and reduced the mean plaque scores but not to a statistically significant level when compared to water (placebo) rinse. There was no significant difference observed when the mean plaque scores were compared between ozonated water and chlorhexidine mouth rinse. However, there was a statistical significant difference in the mean gingival scores between chlorhexidine and ozonated water, with chlorhexidine showing a lower mean score compared to ozonated water. This result was in contrast with previous study conducted by Kshitish and Laxman[11] and Huth *et al*.[20] which shows a higher percentage reduction of plaque (12%) and gingival score (29%), on irrigation with ozonated water, when compared with 0.2% chlorhexidine.

The finding that oral irrigation may reduce plaque score and gingival bleeding score is might due to the possible mechanism of action of oral irrigation like: (1) a change in plaque composition, (2) flushing out of the inflammation inducing factors, or (3) due to flushing effect, the inflammatory mediators in the gingival crevice/pocket, and alter the gingival response to microbial challenge, thereby contributing to gingival homeostasis and thus maintain the gingival tissue integrity.[21,22]

The limitation of this study is that the study did not attempt to evaluate the mechanism of action, as the aim of the study is only to evaluate the efficacy of ozonated water. Furthermore, ozonated water was applied for 1 min. It is known that activity of chlorhexidine is prolonged by its substantivity,[23] but same may not be true for ozone.

Considering the limitations of this study in terms of its short-term duration, ozone can be considered as a promising antimicrobial agent as chlorhexidine to inhibit plaque formation *in vivo*. However, long-term studies of unsupervised use for several months are necessary to establish an inhibitory or therapeutic effect on plaque control and gingivitis to assess fully the adjunctive value of ozonated water when used in conjunction with tooth brushing. It is also required to determine the specific ozone concentration that is effective against periodontal pathogens.

CONCLUSION

The results of the present study demonstrate that chlorhexidine mouth wash was more effective in reducing plaque as compared to ozonated water however, ozonated water and chlorhexidine mouth rinse were equally effective in reducing gingivitis. More studies on ozone therapy should continue to focus on establishment of safe and well defined parameters in accordance with randomized controlled trials to explore the mechanisms involved and also to maintain the gingival and periodontal health for long-term.

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Conflicts of interest
There are no conflicts of interest.

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