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RESEARCH ARTICLE

The Effect of Ozonized Water Irrigation in the Circuits of Professional Ultrasonic Scalers for Causal Therapy of Stage I Periodontitis: A Randomized Clinical Study

Simone Marconcini, Enrica Giammarinaro†, Giacomo Oldoini, and Annamaria Genovesi
Fundationf for Research, Istituto Stomatologico Toscano, Camaiore 55041, Italy

Background: Periodontitis is a chronic inflammatory condition associated with dysbiosis of the oral microbiota. The aim of the present clinical study was to explore the adjunctive effect of ozonized water irrigation in the circuits of ultrasonic scalers for the full-mouth decontamination of patients with periodontitis Stage I or II.
Methods: The study was a randomized, single-blinded, parallel-group clinical trial. The test group (n=25) was treated with ultrasonic scalers irrigated with ozonized water, whereas the control group (n=25) received normal tap water irrigation within the ultrasonic scalers used during the professional mechanical debridement. Full mouth plaque score, bleeding score, probing pocket depth, and the gingival index were evaluated at baseline, two, and 4 weeks after treatment. The pain perceived and dental anxiety were also assessed after treatment by means of the visual analog scale (VAS).
Results: All periodontal parameters resulted in significant improvement for both study groups. The effect of the treatment group on the gingival index was significant, in particular, patients in the test group experienced a greater reduction in this score. No significant differences could be observed with regards to the average probing depth, full mouth plaque index and bleeding score. Patients treated with ozonized water running in the circuits of ultrasonic scalers displayed also lower scores for pain and dental anxiety.
Conclusion: The present study showed a significant clinical effect on gingival inflammation attributable to adjunctive ozone irrigation during nonsurgical periodontal therapy. Further studies, including patients with severe periodontitis and greater sample sizes, are recommended to test the clinical effect of ozonized water in the circuits of ultrasonic scalers.

Key Words: Periodontal disease, Oral hygiene, Ozone

Introduction

Recent advances in molecular sciences and high-throughput technologies for gene sequencing allowed the reclassification of periodontitis as the clinical consequence of chronic dysbiosis of the oral microbiota[1]. Thus, the modern therapeutical approaches are adapting to meet the evolved understanding of the oral environment. Of course, causal therapy, meaning the full mouth decontamination of dental biofilms, still represents the therapeutic gold standard. However, full mouth decontamination per se is unable to cover the entire range of clinical scenarios, and, often, patients or specific periodontal pockets do not respond to traditional therapy[2]. Furthermore, full mouth decontamination has been associated with systemic acute inflammatory response and greater discomfort for the patient[3]. That is why the original protocol by Quirynen et al.[4] has been extensively revised many times over the years by different authors, often with the inclusion of adjunctive chemical agents[5]. Traditionally, chlorhexidine irrigation has been used as the standard antimicrobial therapy to pair the full mouth debridement with[6]. However,

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†Correspondence to: Enrica Giammarinaro, *https://orcid.org/0000-0001-8398-8525*
Istituto Stomatologico Toscano, via Aurelia 335, 55041, Camaiore, Italy
Tel: +39-00393895732709, Fax: +39-0584-6184149, E-mail: e.giammarinaro@gmail.com

chlorhexidine decreases the diversity of the oral microbiota, thus, impairing the resilience of the oral environment to future noxae and disrupting the fitness of the local mucosa-associated immune tissue of the periodontium[7]. Greater attention should be paid to alternative, proactive agents such as the ozone, which is found in nature and has no side effects. Ozone is a highly reactive molecule, with a killing effect on both bacteria and viruses[8]. It has also strong anti-inflammatory, immunostimulatory, immunomodulatory, and analgesic properties[9,10].

Ozonized water in the form of professional oral irrigation has been gaining attention in the last few years. Ozonized water finds many applications in dentistry, including the management of periodontal inflammation, oral mucosa lesions, wound healing, pain, and edema management at surgical sites[11,12]. The aim of the present clinical study was to explore the adjunctive effect of ozonized water irrigation in the circuits of ultrasonic scalers for the full-mouth decontamination of patients with periodontitis Stage I or II when compared to standard tap water irrigation.

Materials and Methods

1. Study design

A randomized, prospective, parallel study design was adopted. Eligible patients were recruited from those attending the Istituto Stomatologico Toscano Clinical Division (Fortis, Forte dei Marmi, Italy) for standard non-surgical periodontal treatment (NSPT). The Unicamillus (Rome) Ethical Committee gave the approval for this study (N 4/2020). The sample size was calculated on the basis of previous literature[13]. The minimum sample size required was calculated as 25 in each group in order to detect a meaningful difference in periodontal inflammation after one month. All patients gave signed an informed written consent.

2. Patient selection criteria

The inclusion criteria were as follows: aged 18 years or older with a minimum of 20 natural teeth; plaque index ≥2.0, modified gingival index (GI) ≥1.5, and modified sulcus bleeding index ≥1.0; Stage I or II periodontitis; at least 3 teeth with periodontal pocket depth ≥4 mm; bleeding on probing >50% of teeth as determined by single-pass probing depth (PD) measurements. Subjects exhibiting 1 of the following criteria were excluded from the study: diabetes; pregnancy or lactation; and having received periodontitis treatment within the 6 months prior to the start of the study.

Those satisfying the inclusion criteria were asked to fill out the anamnestic questionnaire, covering participants' age, sex, tobacco consumption, and continuative drug intake. At baseline, a full dental examination was completed. All eligible participants were assigned a consecutive study number. Patients were randomly allocated to 1 of 2 possible groups of treatment through a computer-generated list.

3. Treatment groups

1) Test group

At baseline, patients allocated to the test group received standardized instructions regarding the home-based disinfection protocol, including the use of sonic toothbrush two times a day, interdental brushing, and tongue cleaning. After 2 weeks, full-mouth non-surgical periodontal treatment (FMNSPT) was performed with the use of ultrasonic scalers with ozonized water irrigation.

2) Control group

At baseline, patients allocated to the test group received standardized instructions regarding the home-based disinfection protocol, including the use of sonic toothbrush two times a day, interdental brushing, and tongue cleaning. After 2 weeks, FMNSPT was performed with the use of ultrasonic scalers with normal tap water irrigation.

4. Follow-up

Patients were evaluated at baseline, and at intervals of one week for one month after NSPT (Fig. 1).

5. Periodontal treatment

In this study, NSPT consisted of the removal of plaque and calculus utilizing sharp smart scaler curettes (Deppeler SA, Rolle, Switzerland) and ultrasonic inserts until the surfaces were hard and smooth. In the test group, the circuits of ultrasonic scalers delivered ozonized water

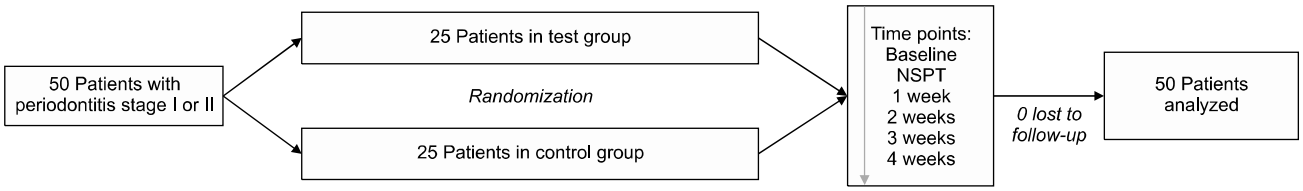

Fig. 1. Flowchart of the experimental timeline. NSPT: non-surgical periodontal treatment.

irrigation during the entire procedure (OzoActive; Mectron s.p.a., Ceresco, Italy). In the case of OzoActive, the production of ozone is continuous, and the average final output of ozone is 70% del of the nominal value (100 mg/h). The aqueous absorption ensures the antibacterial activity of ozone.

6. Clinical assessment

The following clinical variables were recorded at 3 visits by the same blinded, pre-standardized, experienced periodontist. The full-mouth plaque index (FMPI) and full-mouth bleeding on probing (FMBOP) were calculated by transforming the plaque index and the bleeding on probing in full-mouth percentage values by correcting per the numbers of teeth; the GI was assessed as per Löe[14]; PD was recorded to the nearest millimeter using a PCP-UNC 15 probe (Hu-Friedy, City, Milano, Italy). Third molars were excluded.

The primary efficacy endpoint was the reduction in gingival inflammation, between baseline and the other time-points.

7. Psychological assessment

Within 5 minutes after treatment and for the following two days, patients were presented the following question: “How much pain did you feel during the SRP procedure?”. Each patient received a postoperative pain sheet with a visual analog scale (VAS).The VAS used was a continuous scale comprised of a horizontal line, measuring 100 mm in length, anchored by 2 verbal descriptors, 1 for each symptom extreme: “no pain” at the far left (score of 0) and “pain as bad as it could be” or “worst imaginable pain” at the far right (score of 100). The respondent was instructed to mark the point that represented the pain intensity.

8. Statistical analysis

Descriptive, difference and correlation analysis was performed (R version 4.2.0; The R Foundation for Statistical Computing). Each variable of interest was assigned to the appropriate statistical test according to its nature: independent/dependent, continuous/nominal/time-to-event, and normal/non-normal. A repeated-measures ANOVA design was chosen for the factorial multivariate analysis. The inferential statistic was performed using several tests for the relative treatment effects with global or patterned alternatives for the F1-LD-F1 design were applied for testing group (treatment) and time effects, and their interactions. Moreover, pairwise comparisons of the groups, patterned interactions and patterned group effects were tested using this function. Statistical significance was set at 0.05 for all analyses.

Results

1. Demographic details

In total, 50 patients were included in the final analysis, including 22 males and 28 females. No patient was lost during the 1-month follow-up period. The age range was between 25 and 75 years, with a mean of 44.8±13.4 years. Twenty-five patients were analyzed for the control group and 25 for the test group. At baseline, sex and age variables were equally distributed between the two groups. The demographic characteristics of the cohort are presented in Table 1.

2. Clinical outcome

Mean and standard deviations for each parameter explored are presented in Table 2. All clinical parameters significantly improved ($p < 0.001$) over the follow-up period. Fig. 2 report the plots of the relative treatment effect for GI, FMPI, FMBOP, and PD respectively.

Table 1. Study Population Demographic

Patient demographic	Total (n=50)	Test (n=25)	Control (n=25)
Age (y)	44.8±13.9	44.8±16.3	44.8±10.6
Sex (F/M)	28/22	14/11	14/11
Distribution of periodontitis stage (I/II)	20/30	10/15	10/15

Values are presented as mean±standard deviation or number only.
F: female, M: male.

Table 2. Changes over Time in Periodontal Clinical Indicators according to Ozone or Tap Water Irrigation

Outcome	Group	Baseline	1 week	2 week	3 week	4 week
GI	Test group	2.36±0.67	1.09±0.70	0.45±0.68	0.18±0.40	0.36±0.67
	Control group	2.22±0.44	1.78±0.67	1.33±0.50	1.11±0.33	0.88±0.60
$Wald.test			Group statistic: 9.877747, df: 1, $p < 0.05$ Time statistic: 246.948210, df: 4, $p < 0.05$ Group:Time statistic: 40.661739, df: 4, $p < 0.05$			
FMPI%	Test group	0.64±0.24	0.30±0.14	0.17±0.15	0.13±0.19	0.16±0.25
	Control group	0.63±0.24	0.39±0.21	0.19±0.13	0.17±0.15	0.16±0.15
$Wald.test			Group statistic: 0.4860304, df: 1, $p > 0.05$ Time statistic: 199.4111192, df: 4, $p < 0.05$ Group:Time statistic: 9.2745515, df:4, $p > 0.05$			
FMBOP%	Test group	0.51±0.25	0.30±0.18	0.18±0.18	0.09±0.13	0.06±0.11
	Control group	0.48±0.23	0.32±0.18	0.28±0.20	0.16±0.14	0.11±0.11
$Wald.test			Group statistic: 0.7048679, df: 1, $p > 0.05$ Time statistic: 256.5586047, df: 4, $p < 0.05$ Group:Time statistic: 15.3435004, df: 4, $p > 0.05$			
PD	Test group	3.37±0.85				2.14±0.34
	Control group	3.32±0.92				2.46±0.52
$Wald.test			Group statistic: 2.819365, df: 1, $p > 0.05$ Time statistic: 68.816478, df: 1, $p < 0.05$ Group:Time statistic: 7.036979, df: 1, $p > 0.05$			
VAS	Test group	2.13±1.25				
	Control group	3.80±1.32				
$Wald.test			Group statistic: 3.351446, df: 1, $p < 0.05$			

Values are presented as mean±standard deviation.
FMBOP: full-mouth bleeding on probing, FMPI: full-mouth plaque index, GI: gingival index, PD: probing depth, VAS: visual analog scale.

Patients in the test group showed the most benefit from full-mouth NSPT when compared with patients assigned to the control group but without reaching significance, except for GI, which was influenced by the type of treatment. At baseline, the average GI was 2.36±0.67 for the test group and it decreased to 0.36±0.67 4 weeks after treatment. This drop was significantly greater than the one experienced by patients in the control group (baseline GI 2.22±0.44 and 4week GI 0.88±0.60). For the sake of simplicity, we are presenting raw numbers only for the ANOVA test (Table 1).

3. Patient-related outcomes

There was a significant interaction between treatment groups and time on patient-related outcomes (VAS). Patients in the test group showed the lowest scores for pain, dental anxiety, and treatment avoidance over time when compared with the control group ($p < 0.0001$).

Discussion

The results of the present clinical study documented the efficacy of ozonized water irrigation in the circuits of ultrasonic scalers in improving the gingival inflammation

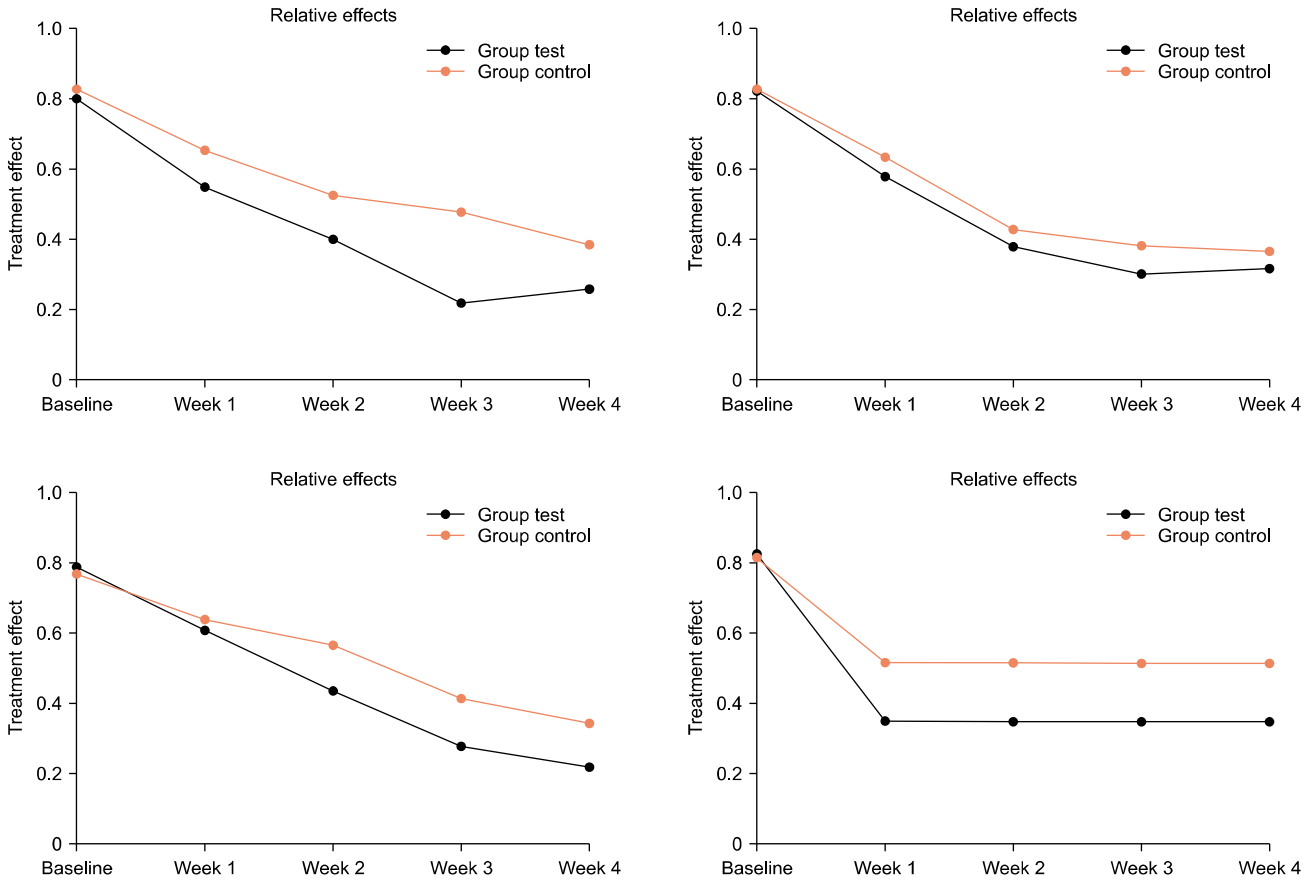

Fig. 2. Plot of the relative treatment effect on gingival index (GI) (A), full-mouth plaque index (FMPI) (B), full-mouth bleeding on probing (FMBOP) (C), probing depth (PD) (D).

of patients with periodontitis Stage I.

The absence of difference in other measures may be due to the main inclusion criteria of the study, which was "patients with a diagnosis of mild periodontal disease". In fact, it is well-known that in order to be detectable, the clinical effect of a periodontal intervention often requires severe cases[14]. However, deep pockets may bias the results, generally exasperating the real effect of the test intervention.

In 2020, the EFP Research Group published an official practice guideline for periodontitis Stage I to III[15]. The authors contemplate the possibility of adjunctive therapies for gingival inflammation in the initial treatment of periodontitis Stage I, II, and III. Among different adjunctive therapies, ozone has gained attention as it targets several needs when it comes to periodontal disease management[16]. Ozone encompasses many biological mechanisms including, immunomodulation, infection and pain control, cell metabolism, and so on[17].

In 2022, Ranjith et al.[13] published the results of a randomized controlled clinical and biochemical study evaluating the adjunctive benefit of ozonized water irrigation with mechanical debridement in the management of Stage III periodontitis. The Authors found that ozone irrigation provided adjunctive benefits along with nonsurgical periodontal therapy in reducing clinical parameters and inflammatory mediators in saliva. In their study, Ranjith et al.[13] had to prepare ozone first by means of an ozone generator and immediately use it for irrigation via blunt-tipped 22-gauge needle at- attached to a 10-ml syringe. In the present study, the production of ozone was continuous, thus ensuring the final concentration of the active molecule and shortening the overall treatment period.

Furthermore, the idea of having a continuous ozonized water supply to the ultrasonic scaler circuits arouse during COVID-19 pandemic, thus, as a consequence of the work-related need of keeping the aerosol within the dental facilities under control[18].

Ozone has a non-indifferent analgesic effect[19], in fact, ozone demonstrated positive effects on blood perfusion measurements during the early healing period of second-intention healing wounds. Blood perfusion to the healing wound is strictly related to the pain perceived.

Even if the determination of the changes in postoperative pain is subjective, in the present study, the VAS values in the control group were significantly higher than those in the test group after NSPT, and so was the treatment avoidance behavior. These findings corroborate those of Kazancioglu et al.[20] who reported that patients in the ozonated group experienced significantly less pain and took fewer analgesic tablets than the control group in the case of third molar surgery.

Ozone does not disturb the diversity of the microbiota and does not have any side effects, thus it might qualify as a proactive means to control periodontal infection[21,22].

Additional studies with larger sample sizes and patient stratification according to periodontal disease severity and associated systemic risk factors would be necessary to confirm the present findings.

In conclusions, the present study supported the clinical efficacy of continuous irrigation with ozonized water within the circuits of ultrasonic scalers during non surgical periodontal treatment of periodontitis Stages I and II. Ozonized water was helpful in reducing gingival inflammation and it was associated with less pain perceived by the patient against standard tap water.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Institutional Review Board of Unicamillus University (N 4/2020).

Author contributions

Conceptualization: Simone Marconcini and Annamaria Genovesi. Data acquisition: Giacomo Oldoini and Annamaria Genovesi. Formal analysis: Enrica Giammarinaro. Funding: Annamaria Genovesi. Supervision: Simone Marconcini and Annamaria Genovesi. Writing–original draft: Simone Marconcini, Enrica Giammarinaro, Giacomo Oldoini, and Annamaria Genovesi. Writing–review & editing: Simone Marconcini, Enrica Giammarinaro, Giacomo Oldoini, and Annamaria Genovesi.

ORCID

Simone Marconcini, *https://orcid.org/0000-0002-7029-138X*
Enrica Giammarinaro, *https://orcid.org/0000-0001-8398-8525*
Giacomo Oldoini, *https://orcid.org/0000-0003-3444-9677*
Annamaria Genovesi, *https://orcid.org/0000-0003-2181-7872*

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Data availability

Data files are available upon request.

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