

## Research Article



# The effects of ozone therapy as an adjunct to the surgical treatment of peri-implantitis

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## ABSTRACT

**Purpose:** The decontamination procedure is a challenging aspect of surgical regenerative therapy (SRT) of peri-implantitis that affects its success. The purpose of the present study was to determine the impact of additional topical gaseous ozone therapy on the decontamination of implant surfaces in SRT of peri-implantitis.

**Methods:** A total of 41 patients (22 males, 19 females; mean age, 53.55±8.98 years) with moderate or advanced peri-implantitis were randomly allocated to the test group (ozone group) with the use of sterile saline with additional ozone therapy or the control group with sterile saline alone for decontamination of the implant surfaces in SRT of peri-implantitis. Clinical and radiographic outcomes were evaluated over a period of 12 months.

**Results:** At the 12-month follow-up, the plaque and gingival index values were significantly better in the ozone group ( $P<0.05$ ). Probing depth decreased from 6.27±1.42 mm and 5.73±1.11 mm at baseline to 2.75±0.7 mm and 3.34±0.85 mm at the end of the 12-month observation period in the ozone and control groups, respectively. Similarly, the clinical attachment level values changed from 6.39±1.23 mm and 5.89±1.23 mm at baseline to 3.23±1.24 mm and 3.91±1.36 mm at the 12-month follow-up in the ozone and control groups, respectively. According to the radiographic evidence, the defect fill between baseline and 12 months postoperatively was 2.32±1.28 mm in the ozone group and 1.17±0.77 mm in the control group, which was a statistically significant between-group difference ( $P<0.05$ ).

**Conclusions:** Implant surface decontamination with the additional use of ozone therapy in SRT of peri-implantitis showed clinically and radiographically significant. Trial registry at ClinicalTrials.gov, NCT03018795.

**Keywords:** Decontamination; Heterografts; Ozone; Peri-implantitis

## INTRODUCTION

Peri-implant diseases are described as inflammatory processes in the tissues surrounding implants in response to predominantly microbial biofilms on the surface of the implants [1]. Peri-implant mucositis is defined as an inflammatory reaction triggered by microbial biofilms based on the parameter of bleeding on probing (BOP), without any loss of peri-implant bone, whereas peri-implantitis is characterized by BOP and/or suppuration with further loss of peri-implant bone [2].

**Conflict of Interest**

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

Since microbial biofilms play a major role in the aetiology of peri-implant diseases, it has been considered that the elimination of microbial pathogens is mandatory for their treatment [3]. The objective of peri-implantitis therapy is to achieve inflammation resolution by decontaminating the implant surface, while preserving the implant-supporting tissues [4].

Several implant decontamination methods have been suggested, including mechanical debridement, chemical therapy (applications of root conditioners, disinfectants, and local and systemic antibiotic therapy) [5,6] and surgical procedures aiming to remove bacteria and to smooth, decontaminate, and detoxify the implant surface [7]. However, there is no consensus on the most effective protocol for implant surface detoxification [8].

Ozone has a strong oxidation effect with remarkable antimicrobial potential, and it can be used as a disinfectant in clinical applications of dentistry [9]. A previous study reported that ozone showed powerful antimicrobial activity in response to anaerobic periodontal pathogenic microorganisms and could have the potential to be used as an adjunctive tool in non-surgical periodontal therapy in periodontitis patients [10]. Ozone therapy can promote haemostasis, enhance the release of growth factors and local oxygen supply, upregulate cellular antioxidant enzymes, and inhibit bacterial proliferation [11]. However, the current literature contains little information regarding the antimicrobial activity of ozone in the treatment of peri-implant diseases. A previous randomized clinical study showed that ozone therapy reduced inflammation in the treatment of peri-implant mucositis [12]. Another *in vitro* trial reported that in the reduction of adherent bacteria on titanium, gaseous ozone showed selective efficacy without any adverse effects on the surface structures of the titanium surfaces or the adhesion and proliferation of osteoblastic cells [13].

Non-surgical therapy alone has been reported to be inadequate for the treatment of moderate and severe forms of peri-implantitis, and surgical therapy is therefore frequently required [1]. The goals of surgical therapy of peri-implantitis are mainly to be able to access the necessary areas for mechanical debridement and implant surface decontamination, to reconstruct anatomic conditions suitable for improving plaque control, and to eliminate pathological peri-implant pockets [14]. This can be achieved through resection or through bone regeneration procedures, such as guided bone regeneration [15].

Studies have evaluated the combination of various regenerative biological agents and techniques for surgical regenerative therapy (SRT) of peri-implantitis, and clinical and radiological improvements following various bone augmentation procedures have been reported [6,14,16-18]. A long-term clinical study demonstrated that vertical peri-implant bone defects (PBDs) may be actively treated by regenerative surgical means, using a bone substitute alone or in combination with a membrane [14].

Platelet concentrates, which include stem cells and growth factors, are preferred in periodontal surgical procedures for the purpose of accelerating angiogenesis, stimulating the activity of osteoblasts and fibroblasts, and obtaining regeneration of hard and soft tissues [19]. These materials can be used alone or in combination with bone grafts or barrier membranes. In studies employing bone grafts with the use of platelet-rich fibrin (PRF) or PRF alone around PBDs, it has been reported that PRF led to increased new bone formation and a higher bone-to-implant contact ratio [20,21]. A recent study reported that treatment of peri-implantitis using PRF was clinically more effective than access flap surgery alone [19].

Various techniques have recently been developed to use platelet concentrate to deliver platelets, leukocytes, growth factors, stem cells, and fibrin matrix at different rates. Sacco developed a therapeutic protocol involving concentrated growth factors (CGF) obtained by separating venous blood through centrifugation using a special device, in the same manner as PRF [22]. Previous studies have reported that CGF accelerated new bone formation in guided bone regeneration treatments [23,24].

The purpose of this randomized, controlled, clinical study was to evaluate the clinical and radiological results of SRT of peri-implantitis following implant surface decontamination using sterile saline alone or in combination with ozone therapy.

## MATERIALS AND METHODS

### Study population and patient selection

The present study was a prospective, randomized, controlled, clinical trial (RCT) reported in accordance with the CONSORT 2010 statement that aimed to investigate the effects of additional ozone therapy for the management of SRT of peri-implantitis over a period of 12 months. Approval for the study was granted by the Institutional Review Board at Gazi University, School of Medicine, Ankara, Turkey (Protocol ID: 25901600-2858). The trial is registered at ClinicalTrials.gov (NCT03018795). Before the procedures, written informed consent was obtained from all participants.

A total of 60 patients were referred to a specialised clinic at the Periodontology Department of Gazi University, with a preliminary diagnosis of a peri-implant infection. Patients in whom peri-implantitis was confirmed by a specialist periodontist (B.U.) were recruited to the study and consecutively treated between November 2014 and October 2016.

The definition of peri-implantitis used in this study was based on the consensus report of the seventh and eighth European Workshop on Periodontology [1,25]. The criteria were the presence of  $\geq 2$  mm of peri-implant marginal bone loss based on baseline periapical radiographs after delivery of the final restoration and BOP and/or suppuration with or without concomitant deepening of peri-implant pockets.

Patients were included in the current study based on the following inclusion criteria: 1) age  $>18$  years, 2) presence of peri-implantitis exhibiting a defect configuration defined as class Ib (buccal dehiscence + semi-circumferential bone resorption), class Ic (buccal dehiscence + circular bone resorption), or class Ie (circumferential bone resorption) [26] with a probing depth (PD) of  $>6$  mm, and 3) no implant mobility and no evidence of occlusal overload. The exclusion criteria were as follows: 1) serious systemic disease that would contraindicate periodontal surgery (i.e., diabetes with a glycosylated haemoglobin level  $>7\%$ ), 2) the requirement of prophylactic antibiotics or systemic antibiotic administration during the past 3 months, and 3) placement and prosthetic loading of implants within the past 1 year.

At baseline, the study included 46 patients in whom at least 1 implant was diagnosed with peri-implantitis. A total of 5 patients failed to attend follow-up appointments and were therefore excluded from the study. The final sample comprised 41 patients (22 males, 19 females; mean age,  $53.55 \pm 8.98$  years). A flow chart of the study participants is presented in Figure 1.

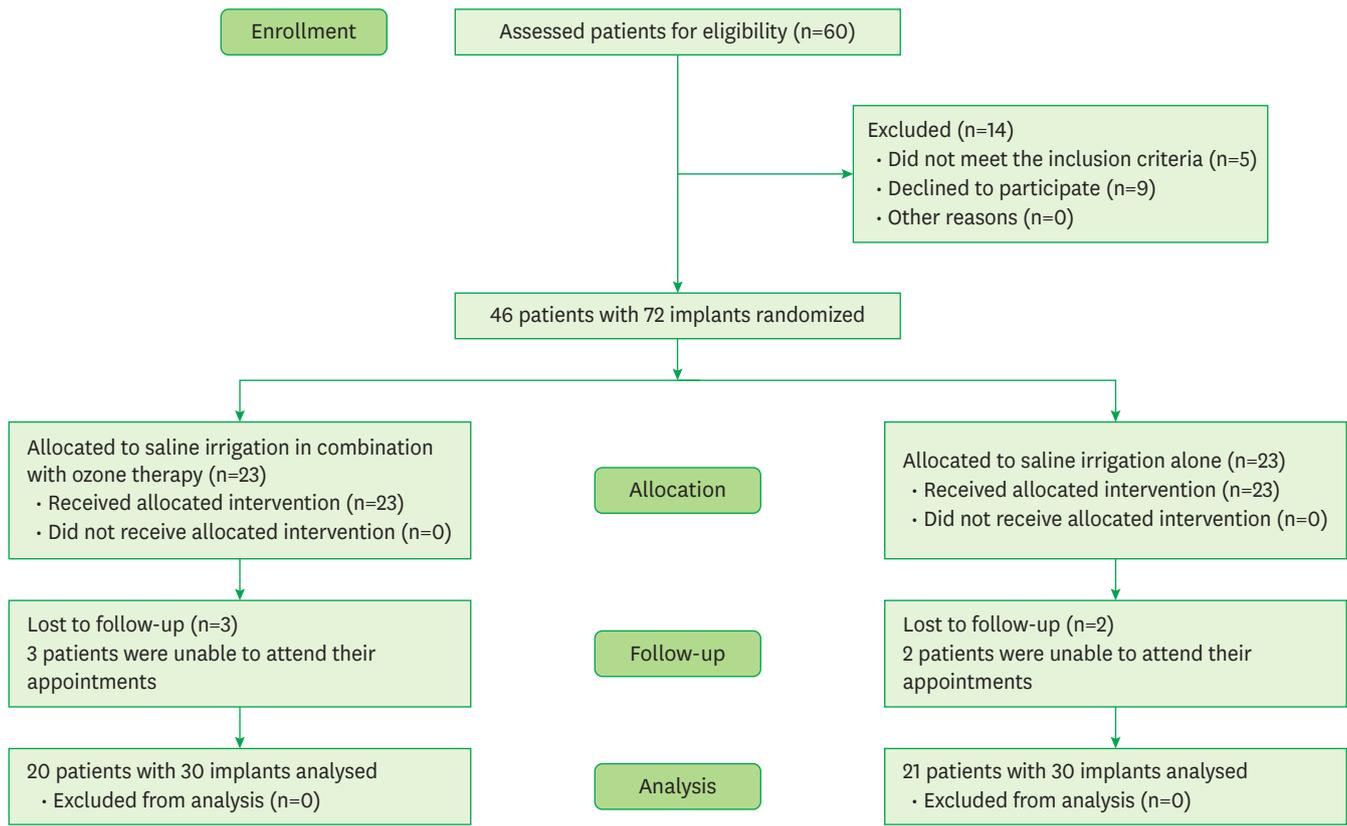


Figure 1. Flow chart of the study participants.

Patients were randomly assigned to either the ozone group (mechanical debridement + decontamination of the implant surface with the use of sterile saline and additional ozone therapy + SRT) or the control group (mechanical debridement + decontamination of the implant surface with the use of sterile saline + SRT) by using a random number generator ([www.random.org](http://www.random.org)). Allocation concealment was achieved using sealed opaque envelopes that assigned the treatment procedure. The randomized treatment code for each patient (ozone or control group) was placed in a closed envelope that was opened prior to the initial therapy.

Professional oral hygiene procedures were applied to each patient. Non-surgical treatment was provided to resolve the inflammation (i.e., suppuration and pus formation) prior to the surgical therapy. For patients with periodontal disease in addition to peri-implantitis, periodontal treatment was applied before the treatment of peri-implantitis. Patients were re-evaluated at least 2 weeks after the initial therapy, and those with a full-mouth plaque score <25% and a full-mouth bleeding score <15% were scheduled for a surgical procedure.

### Clinical and radiographic examination

One examiner (F.S.) assessed clinical parameters with a periodontal probe (N116, Offset, Double End, Color Coded, with Markings, Nordent Mfg Inc., Elk Grove Village, IL, USA). The following clinical measurements were all recorded immediately before surgery (baseline), and at 3, 6, and 12 months after surgery: 1) plaque index (PI) [27], 2) gingival index (GI) [28], 3) BOP [29], 4) PD, 5) mucosal recession (MR), and 6) clinical attachment level (CAL) (PD + MR).

The standardized periapical radiographs were evaluated by 1 examiner (E.P.) who was blinded to the study groups. The periapical radiographs were obtained from each implant using the long-cone paralleling technique with an individualized film holder (Rinn bite film holder for periapical radiographs, Dentsply, York, PA, USA). The film holder was attached at the natural dentition by occlusal fixation using an impression material (Zetaplus, Zhermack, Badia Polesine [RO], Italy). Changes in marginal bone level were evaluated in each implant by comparing radiographs taken during the 12-month follow-up period after the surgical treatment with those taken at the initial clinical examination. The resulting digitized images of the radiographs were analysed using computer software (Viewer, Metasoft, Eskişehir, Turkey). Measurements were taken between the first bone-to-implant contact and a well-defined reference point at the coronal part of the implant body; these points were chosen for the different implant systems at both the mesial and distal aspects of the implants. The vertical defect depth (VDD) measurement was obtained by taking the average of those measurements. The difference in the VDD between the baseline and the 12-month follow-up was defined as defect fill (DF).

Intra-examiner reproducibility of both clinical and radiographic measurements was ensured by selecting 5 implants for the second analysis. The examiners were trained until the coefficient of agreement reached 90%, indicating very good intra-examiner reproducibility. These implants were chosen using a random number table.

### **Implant surface decontamination procedures**

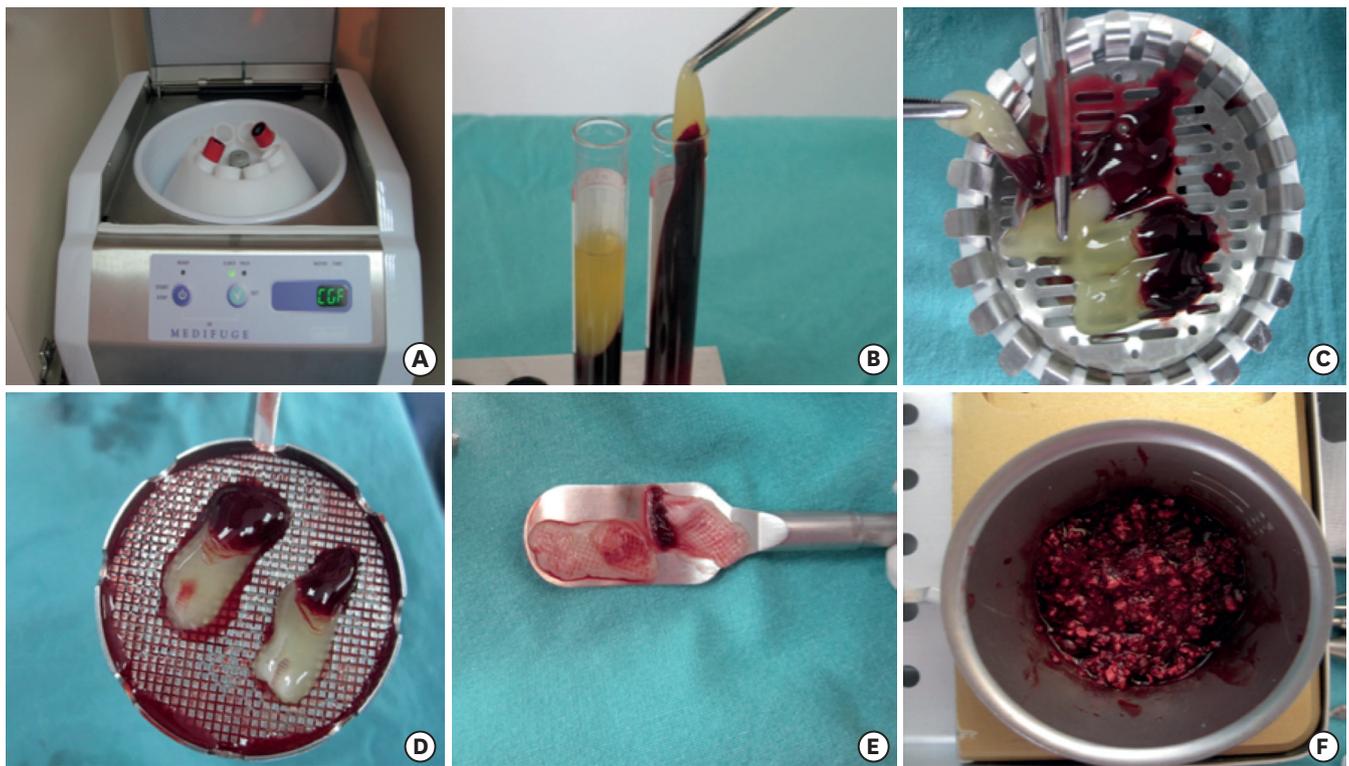
After the non-surgical therapy, the patients were randomly allocated to the groups. At the peri-implantitis sites, mechanical debridement was performed using titanium curettes (ImplaMate, Nordent Mfg Inc.) followed by pocket irrigation with sterile saline for 3 minutes in the control group. Ozone was applied to the peri-implantitis sites following mechanical debridement in the ozone group. The ozone delivery system used was the OzoneDTA Ozone Generator (DentaTec Dental AS, Hov, Norway). According to the manufacturer's information, OzoneDTA produces ozone at a fixed concentration of 2,100 ppm through a connected hand-piece. Ozone was delivered at 6 points circumferentially (2 mesial, 1 midbuccal, 1 midlingual, and 2 distal), for 30 seconds each, using a sterile, specially formed perio-tip. The perio-tip was halted 1 mm short of the pocket depth and ozone was applied as 80% oxygen for 30 seconds per day, 3 days a week, for 1 week. The implant surface decontamination procedures were repeated 3 times, on the starting date of the treatment (day 0), and then 2 days and 4 days later.

### **CGF preparation**

Venous blood samples were obtained and stored in 4 sterile 10-mL tubes without anticoagulants. The blood samples were immediately centrifuged with a CGF centrifuge machine (Medifuge, Silfradent, Santa Sofia, Italy) at 3,000 rpm for 12 minutes. Centrifugation divided the blood into 4 layers, of which the second layer, or buffy coat, and the third layer, also known as the growth factor layer, constituted the CGF. The CGF was squeezed into a special box that produced barrier membranes (Figure 2).

### **Surgical treatment**

All operations were performed by the same specialist periodontist (S.C.I.) under local anaesthesia. Bridges and abutments were removed from the peri-implantitis site, and sulcular incisions around the neck of the implants were then extended mesially and distally to ensure accessibility. To access the PBDs, mucoperiosteal flaps were elevated. Inflammatory tissue was removed from the defect and mechanical instrumentation of the implant surface was performed with titanium curettes. In the control group, instrumentation was followed by irrigation with sterile saline alone (Figure 3),



**Figure 2.** Preparation of CGF. (A) Centrifugation of blood samples with a CGF centrifuge machine. (B) Prepared CGF. (C) Red corpuscles were separated from fibrin clot. (D) Pieces of CGF. (E) CGF barrier membrane. (F) Xenograft mixed with CGF. CGF: concentrated growth factors.

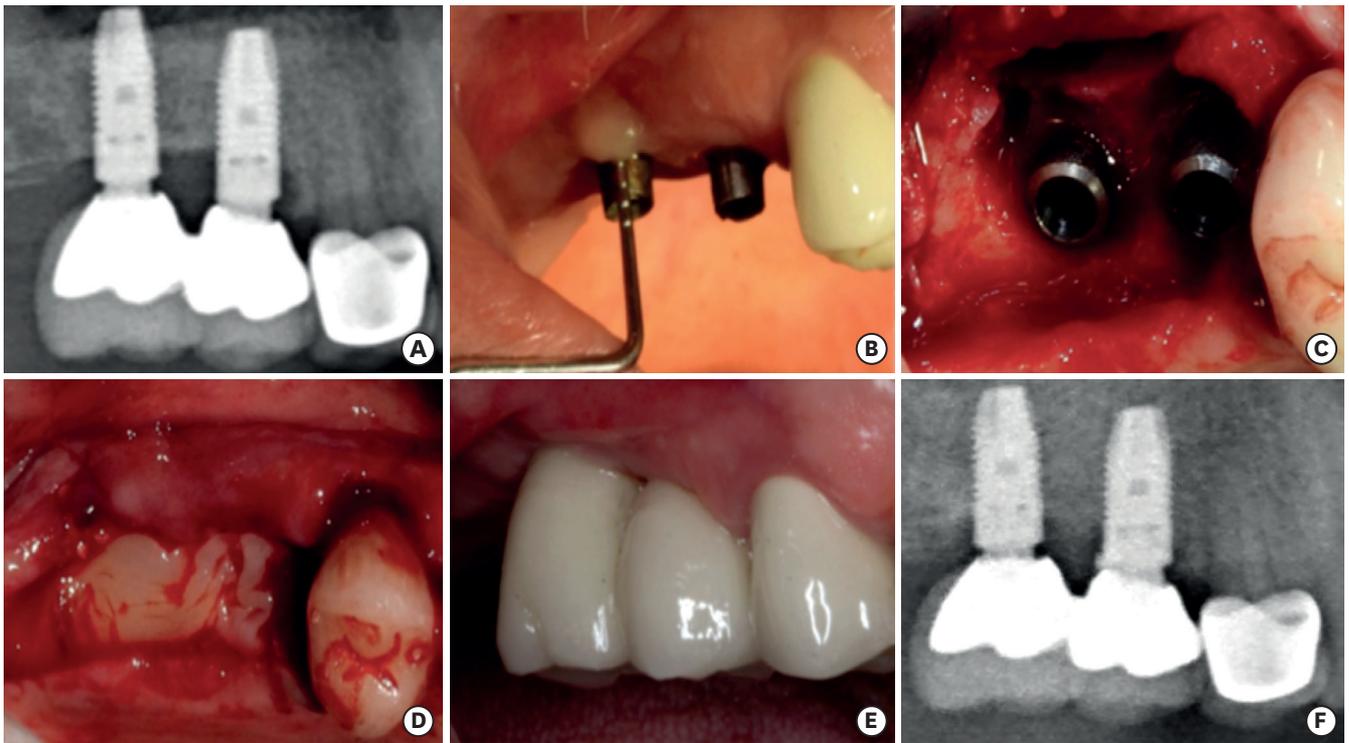
while the same procedure was followed with additional ozone therapy in the ozone group (Figure 4). Ozone was delivered at 6 points circumferentially (2 mesial, 1 midbuccal, 1 midlingual, and 2 distal), for 30 seconds each, using a sterile, specially formed flat probe. The PBDs were filled with a bone substitute (Bio-Oss® spongiosa granules, particle size, 0.25–1 mm, Geistlich, Wolhusen, Switzerland) mixed with small pieces of CGF in both groups. The other 3 pieces of CGF were used as barrier membranes that were placed over the bone substitute. Non-resorbable sutures (Dogsan Surgical Sutures, Trabzon, Turkey) were used for the flap.

The patients were prescribed amoxicillin (500 mg) and metronidazole (500 mg) 3 times a day for 1 week and a 0.12% chlorhexidine mouth rinse twice a day for 2 weeks. All participants were also administered anti-inflammatory and analgesic drugs (flurbiprofen; 100 mg) during the first 3 days. The sutures were removed at approximately 2 weeks after surgery, and a soft toothbrush and approximal brushes were recommended.

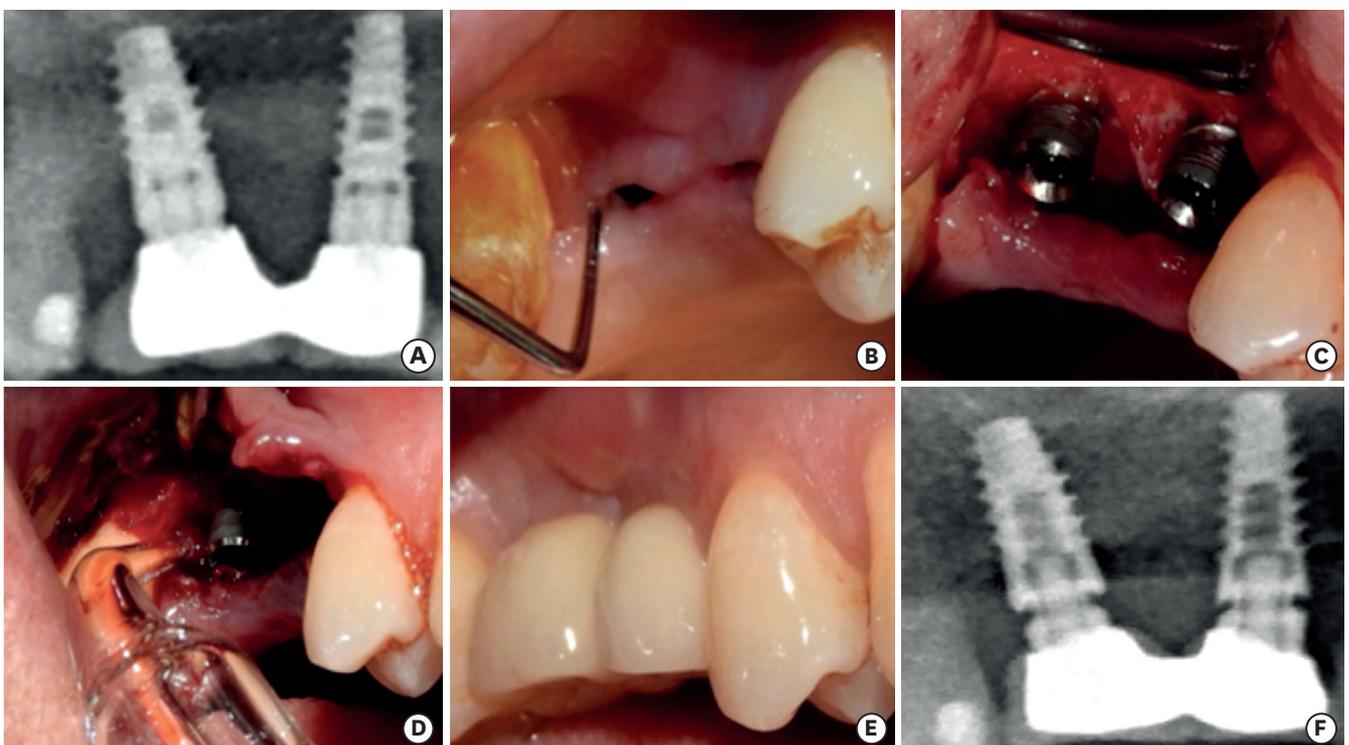
The patients were re-evaluated at 1, 3, 6, 9, and 12 months postoperatively and supportive care was given at the same time points. At 12 months postoperatively, the abutments and bridges were reinstated.

### Outcome

A composite outcome was used to define response to treatment [25]. The treatment was considered to have succeeded (disease resolution) if the implant sites presented with a PD <5 mm without BOP and/or suppuration, no further BL, and DF ≥1 mm as assessed on radiographs at 12 months postoperatively. The primary outcome of this study was DF as assessed by changes in VDD between baseline and the 12-month follow up.



**Figure 3.** Preoperative and postoperative clinical and radiographic views of the control group. (A) Radiographic peri-implant bone defect at baseline. (B) Probing of peri-implantitis site at baseline. (C) Clinical defect configuration after flap elevation. (D) Peri-implant bone defects were filled with a bovine-derived xenograft in combination with concentrated growth factor membranes. (E) Clinical view at 12 months postoperatively. (F) Defect fill at 12 months postoperatively.



**Figure 4.** Preoperative and postoperative clinical and radiographic views of the ozone group. (A) Radiographic view of the peri-implant bone defect at baseline. (B) Probing of peri-implantitis site at baseline. (C) Clinical defect configuration after flap elevation. (D) Implant surface decontamination using ozone therapy. (E) Clinical view at 12 months postoperatively. (F) Defect fill at 12 months postoperatively.

### Statistical analyses

SPSS for Windows version 21.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. The model involved 2-factorial experiments. One of the factors was the treatment method, and the other was the time of the repeated measurements of the patients. Repeated-measures analysis of variance was applied to evaluate the clinical data for treatment methods and time. The Student's *t*-test was performed to evaluate the significance of differences in DF and treatment success between the groups. *Post hoc* comparisons between the groups were made, and the Duncan multiple-range test was used when necessary.

A logistic regression model was used to identify potential patient- and implant-related factors affecting treatment success. Considering the treatment-, patient-, and implant-related factors that potentially affected treatment success in the 12-month observation period after SRT, a model was built to determine which of these factors were statistically significant. Variables with a statistically significant association with the outcome ( $P \leq 0.05$ ) were assessed in the model and their odds ratios (ORs), including 95% confidence intervals, were calculated.

## RESULTS

The study ultimately included 41 patients. In the ozone group, 1 implant disintegrated 6 months postoperatively. During the 6-month observation period, 2 patients in the ozone group and 3 in the control group left the study (Figure 1). The demographic data of the patients and the characteristics of the implants that were assessed are shown in Table 1.

**Table 1.** Demographic data on patients and characteristics of the affected implants

Characteristics	Ozone group	Control group
No. of patients	20	21
Age (yr)	54.4±8.08	54.18±10.36
Sex (female)	9 (45)	10 (47.6)
Current smoking (<10 cigarettes/day)	5 (25)	6 (28.5)
History of periodontitis	9 (45)	8 (38)
No. of implants	30	30
No. of years with a functioning implant	5.15±2.71	4.83±2.14
Type of implants		
Zimmer® (resorbable blast media, blasted with hydroxyapatite surface)	4	5
Adin® (resorbable blast media and SLA surface)	8	7
ITI® (alumina oxide blasted and etched SLActive surface)	2	-
MiS® (acid etching and sandblast surface)	4	5
Astra Tech® (TiO <sub>2</sub> blast + fluoride hydrofluoric acid surface)	5	5
Xive® (acid etching and sandblast surface)	-	1
Lasak® (blasted with hydroxyapatite and SLA surface)	4	3
Nonidentifiable implant systems	3	4
Arch		
Maxilla	15 (50)	14 (46.6)
Mandible	15 (50)	16 (53.4)
Location		
Anterior	8 (26.6)	7 (23.3)
Posterior	22 (73.4)	13 (76.7)
Implant defect characteristics		
Class 1b	8 (26.6)	9 (30)
Class 1c	17 (56.8)	15 (50)
Class 1e	5 (16.6)	6 (20)

Values are presented as mean±standard deviation or number (%).

Data on the clinical and radiographic parameters (mean±standard deviation) at baseline and at 3, 6, and 12 months postoperatively for both groups are presented in Table 2. At baseline, no statistically significant difference was recorded between the groups for any of the clinical parameters. In both groups, a statistically significant difference was found in the PI, GI, and BOP values at 3, 6, and 12 months postoperatively compared to the baseline values ( $P<0.001$ ). At the 12-month follow-up, there were significant differences in the PI and GI values between the groups, in favour of the ozone group ( $P<0.05$ ). Although there was a tendency towards a greater reduction of BOP values in the ozone group, no significant differences between the treatment groups were observed at any time points.

At 12 months postoperatively, significant differences were observed in the PD and CAL values for both groups compared to baseline ( $P<0.001$ ). The PD values decreased from 6.27±1.42 mm and 5.73±1.11 mm at baseline to 2.75±0.7 mm and 3.34±0.85 mm at the end of the 12-month observation period in the ozone and control groups, respectively. The CAL values changed from 6.39±1.23 mm and 5.89±1.23 mm at baseline to 3.23±1.24 mm and 3.91±1.36 mm at the 12-month follow-up in the ozone and control groups, respectively. A significant difference was only detected in the PD values between the groups at the 3-month follow-up period, in favour of the ozone group ( $P<0.05$ ). There were no statistically significant differences in the CAL values between the groups at any time points.

The mean MR increased significantly at 3, 6, and 12 months after treatment ( $P<0.05$ ) in both groups compared to baseline.

**Table 2.** Clinical and radiographic parameters at baseline and at 3, 6, and 12 months postoperatively

Parameters	No.	Baseline	3 mon	6 mon	12 mon	P value
<b>PI</b>						
Ozone group	30	1.21±0.57	0.36±0.47	0.27±0.38	0.22±0.17	<0.001 <sup>a)</sup>
Control group	30	0.96±0.63	0.63±0.64	0.38±0.33	0.49±0.27	<0.001 <sup>a)</sup>
P value		0.765	0.178	0.253	0.047 <sup>b)</sup>	
<b>GI</b>						
Ozone group	30	1.15±0.53	0.15±0.34	0.025±0.1	0.016±0.06	<0.001 <sup>a)</sup>
Control group	30	1.05±0.62	0.29±0.62	0.06±0.19	0.07±0.14	<0.001 <sup>a)</sup>
P value		0.678	0.046 <sup>b)</sup>	0.059	0.01 <sup>b)</sup>	
<b>BOP (%)</b>						
Ozone group	30	96.6±10.85	30±23.1	21.6±27.6	15.8±19.1	<0.001 <sup>a)</sup>
Control group	30	97.5±10.06	28.33±30.06	24.1±26.6	25±21.7	<0.001 <sup>a)</sup>
P value		0.565	0.061	0.546	0.575	
<b>PD (mm)</b>						
Ozone group	30	6.27±1.42	3.05±0.71	2.69±0.81	2.75±0.7	<0.001 <sup>a)</sup>
Control group	30	5.73±1.11	3.44±1.3	3.08±0.97	3.34±0.85	<0.001 <sup>a)</sup>
P value		0.328	0.01 <sup>b)</sup>	0.166	0.158	
<b>CAL (mm)</b>						
Ozone group	30	6.39±1.23	3.53±1.33	3.23±1.48	3.23±1.24	<0.001 <sup>a)</sup>
Control group	30	5.89±1.23	3.84±1.6	3.64±1.44	3.91±1.36	<0.001 <sup>a)</sup>
P value		0.993	0.096	0.946	0.34	
<b>MR (mm)</b>						
Ozone group	30	0.12±0.14	0.4±0.84	0.54±0.88	0.48±0.75	0.01 <sup>a)</sup>
Control group	30	0.25±0.42	0.46±0.57	0.52±0.63	0.55±0.64	0.01 <sup>a)</sup>
P value		0.154	0.221	0.162	0.753	
<b>VDD (mm)</b>						
Ozone group	30	4.63±1.25	-	-	2.54±1.54	<0.001 <sup>a)</sup>
Control group	30	4.23±1.46			3.02±1.57	<0.001 <sup>a)</sup>
P value		0.136			0.294	

PI: plaque index, GI: gingival index, BOP: bleeding on probing, PD: probing depth, CAL: clinical attachment level, MR: mucosal recession, VDD: vertical defect depth.  
<sup>a)</sup>Statistically significant difference compared to the baseline using analysis of variance; <sup>b)</sup>Statistically significant difference between the groups using analysis of variance.

**Table 3.** The percentage of treatment success and mean radiographic DF in the ozone and control groups at the 12-month follow-up

Parameters	Ozone group (n=30)	Control group (n=30)	P value
DF (mm)	2.32±1.28	1.17±0.77	0.02 <sup>a)</sup>
Disease resolution (%)	50	36.6	0.62

Values are presented as mean±standard deviation or percentage.

DF: defect fill.

<sup>a)</sup>Statistically significant difference between the groups using the Student's *t*-test.

In both treatment groups, the VDD values decreased significantly from baseline to the 12-month follow-up ( $P<0.001$ ). Furthermore, according to the radiographic evidence, the DF between baseline and 12 months postoperatively was 2.32±1.28 mm in the ozone group and 1.17±0.77 mm in the control group, which was a statistically significant between-group difference ( $P<0.05$ ) (Table 3). When considering the mean DF values in the present study, with 30 subjects per group, the power analysis revealed a power of 0.98 at a significance level of 0.05 for the Student's *t*-test.

At 12 months postoperatively, 43.3% of the implants showed disease resolution, demonstrating successful peri-implantitis therapy, irrespective of treatment allocation. Treatment success was obtained in 50% of the implants in the ozone group and 36.6% of the implants in the control group. However, a statistically significant difference was not observed between the groups regarding treatment success (Table 3). The factors associated with treatment success were evaluated using multivariate regression analysis (Table 4). The results showed that adjunctive ozone therapy did not have a significant impact on treatment success (OR, 6.601;  $P=0.159$ ), while a history of periodontitis had a negative, but non-significant effect on treatment success (OR, 0.01;  $P=0.47$ ). The implant location (maxilla/mandible)

**Table 4.** Multivariate logistic regression analysis of factors associated with the success of peri-implantitis treatment at 12 months postoperatively

Characteristics	OR	95% CI	P value
<b>Patient characteristics</b>			
Age (yr)	1.03	0.902–1.117	0.658
Gender			
Female <sup>a)</sup>			
Male	0.273	0.17–4.302	0.356
Smoking (<10 cigarettes/day)			
No <sup>a)</sup>			
Yes	0.34	0.01–1.586	0.084
History of periodontitis			
No <sup>a)</sup>			
Yes	0.001	0–0.92	0.47 <sup>b)</sup>
<b>Implant characteristics</b>			
No. of treated implants per patient			
n=1 (single) <sup>a)</sup>			
n>1 (multiple)	1.172	0.351–3.91	0.797
Implant function time	2.31	0.672–7.943	0.184
Implant location			
Mandible <sup>a)</sup>			
Maxilla	0.22	0.01–0.705	0.031 <sup>b)</sup>
Anterior region <sup>a)</sup>			
Posterior region	0.435	0.005–40.5	0.719
<b>Treatment factors</b>			
Ozone therapy			
No <sup>a)</sup>			
Yes	6.601	0.478–91.096	0.159

OR: odds ratio, 95% CI: 95% confidence interval.

<sup>a)</sup>Reference category; <sup>b)</sup> $P<0.05$  considered statistically significant for the multivariate logistic regression analysis.

variable was found to be statistically significant (OR, 0.022;  $P=0.031$ ), with implant location in the maxilla associated with a positive effect on treatment success.

## DISCUSSION

Peri-implantitis is a challenging biological complication of dental implants, for which there are no commonly agreed protocols with predictable treatment outcomes. The surgical treatment of peri-implantitis with implant surface decontamination is recommended because decontamination eliminates the microbial biofilm from the surface of implants, yielding a surface compatible with health and allowing re-osseointegration [4].

Implant surface decontamination is a major challenge in the procedure of treating peri-implantitis. In a canine model of peri-implantitis, Parlar et al. [30] assessed the effects of various implant decontamination methods and surface configurations on the re-osseointegration of contaminated implant surfaces. The highest bone-to-implant contact was found on implant surfaces that were sprayed with saline *in situ*. Berglundh et al. [31] reported that the local use of antiseptic agents, air abrasives, or lasers for decontamination of the implant surface in surgical therapy of peri-implantitis did not improve treatment outcomes compared with mechanical debridement combined with topical saline.

In recent years, ozone therapy has been suggested as a new adjunctive treatment strategy for the management of periodontal disease [9]. Significant improvements in PD, PI, GI, and bacterial count in quadrants treated with ozone therapy as an adjunct to non-surgical periodontal treatment have been reported in studies of chronic and aggressive periodontitis patients [9,32,33]. However, the current RCT evaluated the impact of additional ozone therapy on implant surface decontamination in SRT of peri-implantitis. To the best of our knowledge, this is the first time that ozone therapy has been used clinically for implant surface decontamination in peri-implantitis treatment. Mckenna et al. [12] showed that gaseous ozone with or without hydrogen peroxide reduced plaque and bleeding scores around the soft tissues in patients with peri-implant mucositis. Their study also reported that the antimicrobial effect of ozone therapy could delay the advancement of peri-implant diseases, thereby preventing soft tissue friability and providing better soft tissue handling if surgical therapy is necessary. In this study, the treatment modalities in both groups led to significant clinical improvements, as evidenced by reductions in PI, GI, BOP, PD, and CAL values. Our findings showed that adjunctive ozone therapy significantly reduced the mean PI and GI values compared to saline irrigation alone in SRT of peri-implantitis. However, no statistically significant differences were observed between the 2 groups for BOP values; BOP improved to a greater extent in the ozone group, but the discrepancy was not significant.

SRT has been reported to result in a healthier clinical environment around treated implants [34]. A recent meta-analysis revealed that PD was reduced by 2.78 mm after SRT of peri-implantitis, with reductions observed in all included studies [15]. In the current study, the PD reduction was 3.52 mm in the ozone group and 2.39 mm in the control group at the end of a 12-month observation period. Accordingly, a significant reduction was also noticed in CAL values in both groups in the present study. The CAL values improved by 3.16 mm in the ozone group and 1.99 mm in the control group. The only significant difference between the groups was seen in PD values at 3 months postoperatively ( $P<0.05$ ). This finding indicates that adjunctive ozone therapy was superior compared to the control treatment regarding PD values

in the short-term results. However, the ozone group did not show a statistically significant difference in CAL values compared to the control group at any time points. Furthermore, previous RCTs indicated that implant surface decontamination methods did not significantly affect the clinical results of surgical peri-implantitis treatment [7,35]. In fact, as reported by Schwarz et al. [36], in a long-term evaluation of surgical therapy of peri-implantitis, the method used for decontamination seemed to have a small impact on treatment success; moreover, the long-term outcomes of surgical therapy of peri-implantitis could be influenced by factors other than the surface debridement and decontamination methods.

Previous studies have shown that radiologic marginal bone level increased after an anti-infective protocol combined with SRT of peri-implantitis [6,14,37,38]. Comparable results regarding DF were obtained in the current study. In this study, bovine-derived xenograft was used to fill PBDs, and the DF was determined to be  $2.32 \pm 1.28$  mm in the ozone group and  $1.17 \pm 0.77$  mm in the control group at 12 months postoperatively. Our results of the control group compare favourably with the study reported by Aghazadeh et al. [38], where the use of bovine-derived xenograft in PBDs led to  $1.1 \pm 0.3$  mm of DF at a 12-month follow-up. It was also noted in their study that bovine-derived xenograft provided more radiographic DF than autologous bone graft in SRT of peri-implantitis [38]. As demonstrated by the present study, the addition of gaseous ozone therapy to SRT of peri-implantitis yielded enhanced radiographic evidence of DF at 12 months postoperatively. Although no comparative human studies exist evaluating the effect of ozone therapy on bone augmentation procedures, Ozdemir et al. [11] reported that ozone therapy increased bone formation in autogenous bone grafts in a rat calvarial defect model. In another animal-model study, Alpan et al. [39] stated that topical gaseous ozone therapy enhanced bone regeneration in diabetic rat calvarial defects treated with xenografts.

Surgical regenerative techniques for peri-implantitis techniques using various modalities have been described, including polytetrafluoroethylene (PTFE), collagen, synthetic membranes, or no membrane placed over the bone substitute [15]. The benefits of using barrier membranes in peri-implantitis treatment are not clear [15]. In a study by Khoury and Buchmann [37], the outcomes of 3 different modalities for SRT of peri-implantitis were evaluated. Autologous bone graft alone, autologous bone graft in combination with a non-resorbable membrane, or autologous bone graft in combination with a resorbable membrane was used to fill PBDs in their study. Healing complications were observed in the treatment groups in which a barrier membrane was used, and the PD in implants treated with autologous bone graft alone was determined to be 5.4 mm at 12 months, whereas that in the group with an additional ePTFE membrane was 4.8 mm and that in the group with an additional collagen membrane was 3.3 mm [37]. Roos-Jansåker et al. [14] reported that the use of additional barrier membranes made no significant improvement in the outcomes at a 5-year follow-up. PD decreased in implants treated with bone substitute alone to  $3.0 \pm 2.4$  mm, in comparison to  $3.3 \pm 2.09$  mm in the group where a resorbable membrane was also used [14]. It was concluded in a recent systematic review that using a barrier membrane is expensive, time-consuming, and technique-sensitive, with a risk of healing complications (e.g., membrane exposure), and it has no additional benefit on DF [15].

There is little information in the literature about the effect of PRF treatment on PBDs. The effect of PRF application on peri-implant defects has been evaluated in animal models, and those studies have shown that PRF application alone or in combination with silk fibroin achieved new bone formation in  $29.30\% \pm 7.5\%$  and  $51.93\% \pm 27.9\%$  of the subjects, respectively [20,21]. Hamzacebi et al. [19] evaluated the clinical efficacy of flap surgery with

PRF application and flap surgery alone in the surgical treatment of peri-implantitis. PRF application in peri-implant bone loss was determined to provide better clinical results than flap surgery alone. In the current study, the PBDs were filled with CGF in combination with bovine-derived xenograft, and CGF membranes were placed over the bone substitute. Similar to the effect of PRF treatment on peri-implantitis defects in previous studies, CGF application in peri-implantitis treatment in the current study yielded successful clinical results, especially in terms of soft tissue healing.

Disease resolution has been suggested to be an efficient composite therapeutic endpoint for evaluating the treatment success of surgical management of peri-implantitis [25]. In the present study, the effects of possible factors related to treatment methods and patient and implant characteristics on disease resolution were evaluated in a multivariate logistic regression model. Depending on the analysis, history of periodontitis and location within the jaw had significant impacts on treatment success. However, both these variables had comparable distributions in the treatment groups in the current study. Thus, it can be assumed that these variables did not affect the difference in treatment success between the groups.

The distribution of different implant systems and surface characteristics in the ozone and control groups can be considered as a limitation of the present study. However, no data from RCTs have been obtained about the influence of surface characteristics on the response to healing in grafting procedures performed as part of regenerative treatment in association with the decontamination method. However, in an *in vitro* study, gaseous ozone showed successful antimicrobial activity on various titanium and zirconia surfaces without causing any surface damage [13].

In the present study, the results showed clinically and radiographically significant improvements in SRT of peri-implantitis in combination with ozone therapy. Although topical gaseous ozone therapy led to significantly more radiographic evidence of DF in SRT of peri-implantitis, it is difficult to recognize the graft material and newly formed osseous tissue without a histologic evaluation. In the current study, there was no evaluation of the effect of ozone application in peri-implantitis treatment on different biological parameters and microbial species. Further studies are needed to clarify these issues.

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