The treatment of periodontal disease using local oxygen-ozone

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Abstract

The objective was to clinically and microbiologically evaluate local oxygen-ozone therapy used in combination with traditional mechanical therapy *versus* the use of mechanical therapy alone in a group of patients with periodontal disease. To date, this study is the most representative investigation in the scientific literature as it has the largest sample (113 patients). The project was fully completed in the planning phase, the experimental research stage, the statistical analysis phase and the scientific article draft phase. The results of the study showed a sharp reduction in the clinical-microbiological parameters measured for both study groups. In particular, subjects in the group treated with oxygen-ozone therapy plus mechanical therapy showed a more marked improvement. Although it is possible to observe a general trend of improvement in oral hygiene in Western countries, periodontal health remains a goal yet to be achieved in many subjects. In Italy, roughly 60% of the population suffers from periodontal disease (from mild to severe) and roughly 10-14% exhibit fairly advanced forms. The latter group increases dramatically in the age group between 35 and 44 years. The main objective of the clinician who is faced with a patient with periodontal disease is to adopt an appropriate therapeutic approach to halt the progression of the disease and to prevent or reduce the occurrence of any relapses. Mechanical causal therapy is the treatment of choice. However, due to the early recolonization of periodontal pockets by bacteria, various studies are underway in order to find a valid aid to mechanical therapy. In this regard, oxygen-ozone therapy may be an economic, non-invasive and easy method to implement in the clinical management of the patient with periodontal disease. One immediate application is the opportunity to use the treatment in selected patients, such as those who present a recurrence of the disease. For example, systemic antibiotic therapy could be replaced with local oxygen-ozone therapy in patients with periodontal abscess. However, additional longitudinal studies are needed to assess any exact range, timing and method of application.

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Introduction

Periodontal disease (PD) is a chronic infectious-inflammatory disease with a multifactorial cyclic evolution, characterized by the destruction of the fibers of the periodontal ligament, resulting in the formation of pockets, apical migration of the junctional epithelium, alveolar bone loss and, in the more advanced stages, marked mobility eventually leading to tooth loss due to lack of support.

Elevated levels of anaerobic bacteria specific to PD and present in dental plaque, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Treponema denticola* and *Fusobacterium nucleatum*, are the primary etiologic factor in PD.[1,2] In fact, it is now known that the tissue destruction observed in PD is the result of the damaging effect of the host immune response to the action of pathogenic bacteria.[3]

Eliminating or at least reducing the loads of these bacterial pathogens is the primary purpose of PD treatment.

Mechanical periodontal therapy (MPT) is the treatment of choice for periodontal diseases, and is defined as the *gold standard* therapy against which other therapies are compared to prove their effectiveness.[4-10] Numerous studies have shown the beneficial effects of this treatment both from a clinical point of view and from a microbiological point of view.[11-16] MPT is able to remove the supra- and subgingival bacterial plaque and tartar deposits attached to the surface of the tooth.[17,18] Clinically, this means a reduction in probing depth and the bleeding on probing index.[14-16] Numerous clinical and microbiological studies have shown that MPT also causes a decrease in the total bacterial load and a shift in the subgingival microflora toward less pathogenic species more similar to those in healthy periodontium.[11,12] Most of the beneficial effects occur within the first 3 months of treatment, followed by a period of clinical stability that is strongly influenced by the patient's home oral hygiene.[19] Post-therapeutic bacterial recolonization of the root surface by periodontal pathogens is a frequent occurrence, often resulting in recurrence of the disease with further destruction of the periodontal tissue.[20-22] Several factors are involved in bacterial recolonization after mechanical therapy, such as the presence of deep pockets, furcations, or bacterial reserves in niches in the oral cavity, such as the tongue, the tonsils, the periodontal tissues.[23,24]

Due to early bacterial recolonization, over time new therapeutic approaches (surgical and non-surgical) have been proposed as alternatives to conventional MPT or, more often, in combination with the latter.

Hyperbaric oxygen therapy (HBO) has been seen to be successful in various medical applications.[25-27] In general, an increase in the amount of oxygen-ozone available speeds up the healing process in the soft and hard tissues in all parts of the body.[28-32] It is necessary to distinguish the direct effect on bacterial agents from the effect supporting the defense mechanisms and the regeneration of the organism.[30,31,33] Thus, in infections caused by anaerobic microorganisms, the increase in the portion of oxygen can create an environment that is not suitable for the replication of anaerobic bacteria.[34,35] In addition, oxygen has a vasoconstriction action with edema reduction, anti-inflammatory and anti-reactive action, facilitates capillary vascular proliferation and the revascularization of ischemic areas. The benefits of oxygen-ozone on the tissues are manifested through encouraging collagen production, promoting the replication of fibroblasts, stimulating white blood cell function and metabolism and bone turnover. Oxygen also depresses the cell-mediated immune response, alters the prostaglandin equilibrium, protects tissues from the damage caused by the phenomenon of ischemia/reperfusion maintaining normal levels of ATPase, phosphocreatine kinase and low levels of lactate, it protects membranes from free radical lipid peroxidation, it inhibits the production of 2-integrins that favor the adhesion of leukocytes to the capillary wall, resulting in endothelial damage.[28,34-38]

Frequently, oxygen is centrally applied, increasing the oxygen tension in the air breathed during HBO treatment sessions. This leads to an increase in the amount of ozone-oxygen physically dissolved in the blood and, therefore, to higher levels of oxygen in the peripheral regions. Intact skin is a barrier to the direct diffusion of oxygen, therefore the central application of oxygen is the only way to increase the oxygen tension in the affected region. In cases of superficial wounds with compromised integrity of the skin or mucosa, external local applications may be used in place of central oxygen therapy.

The hypothesis of using HBO in the treatment of PD is based on the fact that the oxygen-ozone concentration is lower in deep periodontal pockets,[39] and this could encourage faster colonization of the pockets by periodontal pathogens.[40-42] The surface tension of oxygen (pO_2) in normal tissue is 30-40 mmHg, but in ischemia caused by infections, trauma or edema, the oxygen-ozone levels are much lower. Under 30mmHg, the functions of fibroblasts and white blood cells are severely compromised. When hyperbaric oxygen is applied,[37] the pO_2 can reach 250-300 mm Hg, achieving the beneficial effects mentioned above. Experimental studies have shown that oxygen-ozone therapy may have a beneficial effect in patients with periodontitis, mainly through a dual mechanism: inhibiting the growth of anaerobic bacteria in periodontal pockets[43,44] and increasing blood flow to the gingival level thus promoting tissue healing.[45] In addition, oxygen-ozone stimulates white blood cell function by activating host defense mechanisms and accelerating the healing process.[33] The repair processes are also favored by an increase in tissue capillarity and stimulation of fibroblast replication.[45]

The positive effects of HBO in the treatment of PD have already been evaluated positively, with interesting results in the short to medium term.[46-50]

By contrast, there is very little literature regarding the antimicrobial effect of locally applied oxygen on periodontal pathogens.

As early as 1935, and subsequently in 1955, Box successfully proposed the use of oxygen-ozone insufflation for the treatment of PD.[51,52] Nowadays, after the crucial role of microorganisms has been proven scientifically,[41,53-55] it can be understood that the positive results of the Box empirical method can be linked to the direct exposure of periodontal pathogens to high oxygen-ozone levels inside the periodontal pockets.[39]

More recently, Gaggl and associates (2006)[44] demonstrated the effectiveness of local oxygen-ozone therapy in a sample of patients with acute necrotizing periodontitis. In patients treated with the addition to traditional therapy (oral hygiene, drug therapy) of oxygen-ozone, administered through facemasks, there was a marked reduction or the complete elimination of the periodontal microorganisms evaluated, resulting in a more immediate clinical recovery and therefore less periodontal destruction.

The aim of this study was to evaluate the clinical and microbiological effectiveness of local oxygen-ozone therapy in the treatment of subjects with chronic PD.

Materials and Methods

A prospective randomized clinical study was conducted on a group of 113 subjects with PD. This research project was conducted at the Institute of Clinical Dentistry at the University of Sassari, and was made feasible thanks to the support of the Autonomous Region of Sardinia through a research grant co-financed with funds from the ESF Operational Programme for Sardinia 2007-2013, L.R.7 / 2007 *Promotion of scientific research and technological innovation in Sardinia*. This study was approved by the Ethics Committee of the University of Sassari. The inclusion of each patient in the study was subject to the acquisition of written informed consent.

Sample selection

The number of PD patients who came to the periodontology clinics of the Dental Clinic of the University of Sassari was respectively 181 and 214 in 2010 and 2011. The sample size was calculated using *power analysis* (Point biserial model) in order to obtain a power of 95% with an alpha of 0.05 and a beta power (1-beta errprob) of 0.95. Thus, the theoretical minimum number of subjects for each group was derived as 51 persons.

The inclusion criteria for the study were: presence of PD with at least eight teeth with pocket depths >5 mm; at least 20 teeth in the mouth, as indicated by the WHO; age over 30 years. Not considered eligible for the study were subjects who were suffering from systemic diseases such as cardiovascular diseases, cancer, diabetes or chronic infections; pregnant or breastfeeding; with physical or mental disabilities that could interfere with proper oral hygiene; wearers of fixed or removable orthodontic appliances; who had undergone session(s) with hand or mechanical instruments in the previous six months; had been on antibiotic therapy in the six months preceding the study and/or anti-inflammatory drugs in the previous six weeks; had used mouthwashes in the six weeks prior to the study.

Enrolled patients were included in a list using a *spreadsheet* (Microsoft Excel 2010) and individual randomization was performed. Two groups were formed: one group received mechanical treatment + local oxygen-ozone therapy (case group) and a control group received mechanical therapy alone.

Methods

During the patient's first visit, the following procedures were performed: verification of inclusion criteria, acquisition of written informed consent, filling out an *ad hoc* questionnaire, periodontal examination and selection of sites for microbiological sampling.

The collection of information in the questionnaire is divided into three general sections: i) patient demographics-vital statistics; ii) risk factors (general health, medications, smoking habits); iii) oral health (oral hygiene habits such as the frequency of brushing and the use of auxiliary tools, frequency of dental check-ups).

Periodontal status was evaluated based on the presence of bleeding on probing, and the depth of the gingival pocket. Bleeding on probing was defined positive when the site presented bleeding, including spot bleeding, no more than 20 seconds after removal of the tip of the periodontal probe.[56] The bleeding on probing (BOP) index was calculated as the percentage ratio between the number of bleeding sites and number of sites probed.

The probing depth for each tooth was measured in millimeters using a periodontal probe PCP UNC 15 (Hu-Friedy, USA) at 4 points around each tooth (mesial, distal, buccal, lingual-palatal) as the distance from the free gingival margin to the attachment of the periodontal ligament.[57] The probe was kept parallel to the long axis of the tooth in the vestibular and lingual sites, while in the proximal areas it was positioned as close as possible to the interdental point of contact and slightly angled in order to determine the maximum apical depth of the pocket. The third molars were excluded from the assessment.

All measurements were performed by a single operator who was unaware of the allocation of the patients themselves. Operator standardization was carried out 40 days before the beginning of the recruitment phase: 20 patients with chronic PD were examined and reassessed 72 hours later. Intra-operator reliability, calculated with Cohen's kappa coefficient based on PD, was 0.85.

Microbiological analysis

A sample of subgingival plaque was taken from 4 sites (with PD value ≥ 5 mm) identified for each patient. This site was tested both at baseline and at the subsequent follow-ups. The plaque samples were collected according to a standardized procedure[58] and following the specific recommendations for the diagnostic test used (Meridol® PerioDiagnostics, GABA Münchenstein, Switzerland). In the vicinity of the sample site of collection, the saliva was absorbed with gauze or a cotton roll; subsequently, the plaque was collected by inserting a sterile paper cone 40.02 into the gingival sulcus which was left in place for about 15 seconds. The 4 samples obtained were stored in the same sterile container at room temperature up to the time of shipment to the laboratory (CarpegenGmbH, Münster, Germany), and processed together to obtain a patient-specific microbiological evaluation. A quantitative assessment was performed (Real Time-PCR) of the bacterial load of the following periodontal bacteria: *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Fusobacterium nucleatum* (Fn) and *Prevotella intermedia* (Pi). The threshold value of the microbiological test for the identification of microbial species listed was 10^2 bacteria/plaque sample.

Treatment

Each patient was encouraged and taught how to perform home oral hygiene (toothbrush use with personalized techniques, use of interdental cleaning tools).

Patients subsequently received causal periodontal treatment under local anesthesia (*scaling* and *root planing* within 48h), and oxygen-ozone therapy in the case group (for ten consecutive days).

The oxygen-ozone therapy protocol implemented has been described by Gaggl and associates.[44] For this purpose a mask was constructed made of silicone, with a front valve for oxygen-ozone. The mask provides good adhesion to the patient's mucosa to allow the oxygen-ozone to make good contact with the gingival tissue (Figure 1). The treatment was performed once a day for ten consecutive days with an ozone-oxygen flow of 5 L/min for fifteen minutes.

Clinical parameters and bacteriological samples for each patient were collected at baseline (t_0), after 6-8 weeks (t_1) and 6 months (t_2) after the last root planing session.

Data analysis

The data was coded and entered into a spreadsheet (Microsoft Excel). Before statistical analysis, the absolute values of the bacterial loads were categorized into 4 classes: 0 absent; 1<10^5; 2=10^5; 3≥10^6. The statistical analysis was performed with Stata 9.1 software. A descriptive analysis of the sample was performed. The statistical analysis was performed in order to assess statistically significant differences (P<0.05) between the two groups in question with regard to the indices of periodontal health and the microbial load. This allows the variations in the microbial load for the periodontal pathogens and the periodontal indices to be quantified in a sensitive manner, in order to assess any therapeutic effect of oxygen-ozone therapy. The association between clinical variables and categorization in cases or controls depending on the treatment, was tested with the use of the chi-square test. The differences in the periodontal conditions in the different stages of the study were evaluated with two-way analysis of variance.

Results

A total of 153 PD patients fell within the inclusion criteria for participation in the study; of these patients, 18 did not agree to take part in the study and 22 patients did not complete the study. Consequently, this study shows data for 113 subjects.

The 113 subjects were divided randomly into two study groups: 56 subjects (25 males, 31 females; mean age 43.30±11.54) received mechanical therapy + local oxygen-ozone therapy (case group) and 57 subjects (17 males and 40 females; mean age 45.81±8.36) received MPT alone (control group). 60.71% (n=34) of subjects in the case group and 50.88% (n=29) of subjects in the control group were smokers. Almost all of the sample (107 subjects; 94.69%) were Italian. 21.43% (n=12) of subjects in the case group and 31.58% (n=18) in the control group had a higher diploma or degree as educational qualification. All patients had already undergone dental treatment previously, but as much as 62.50% (n=35) and 54.39% (n=31) of patients respectively in the case group and control group only went to the dentist when they were experiencing pain.

At baseline, the PD mean±standard error (SE) in the case group and the control group were 4.13±0.66 and 3.98±0.60 respectively. The mean BOP (±SE) was 89.90±1.66 [95% CI 86.57-93.24] in the case group and 86.66±1.86 [95% CI 82.92-90.40] in the control group. At baseline the difference between the different clinical parameters and demographic-behavioral factors was comparable between the two groups.

The clinical effects of periodontal treatment can be seen in Table 1 and in Figures 2-5.

Six to eight weeks after root planing (t_1), there was a marked reduc-

Figure 1. Application of the oxygen-ozone via a mask to the maxillary arch.

tion in the clinical parameters considered in both study groups. The average PD was reduced to 3.3±0.3 and 3.5±0.4 in the group case and the control group (P<0.01) respectively. In particular, it was observed in both groups that the deepest pockets (PD>6mm) had the greatest decrease. Intermediate values (PD=5-6 mm) had a moderate improvement, while pockets of 4 mm on probing had minimal changes. The average BOP showed a significant reduction in both groups [16.52±1.77 (95% CI 12.96-20.08) in the case group; 21.87±1.45 (95% CI 18.96-24.77) in the control group, F=4.83 P=0.03 between BOP t_1 and BOP t_0]. Furthermore, the difference Δ between t_1 and t_0 is statistically significant in the two study groups (P<0.01); this did not prove to be significantly associated to any demographic variable or habit, with the exception of the frequency of dental visits, which is associated with the case group (P<0.01).

Six months after root planing (t_2), the results were quite different in the two groups. Subjects who received oxygen-ozone therapy as an integral part of the periodontal treatment had significant stabilization of the clinical values. The average PD had a value of 3.5±0.5 in the case group and 3.6±0.3 in the control group. However, the average BOP had a slight increase in the values (case: 16.95±2.22 [95% CI 12.50-21.40] and control: 26.65±1.84 [95% CI 22.97-30.34] F=0.05 P=0.82 for t_1 and F=5.82 P=0.02 for t_0). In this case too, the differential Δ between t_2 and baseline is significantly associated only to the frequency of dental visits in the case group (P<0.01). The difference Δ between t_2 and t_1 and between t_2 and t_0 is not, however, statistically significant within the two study groups.

The Anova model constructed showed high significance for the variation in the average BOP between the 2 groups in the various times examined (F=5.70, P<0.01).

The levels of pathogenic periodontal bacteria examined at baseline and two follow-up times are shown in Figures 4 and 5. At baseline, both of the study groups showed high levels of periodontal pathogens. At t_1 reductions were observed for all periodontal bacteria, mainly in the group also treated with oxygen-ozone. These differences appear to be highly variable from patient to patient, and only very few patients had complete eradication of the bacteria. At t_2 the microbiological load of the pathogenic bacteria rose compared to the values for t_1. The statistical analysis between the different bacterial loads at different times showed a statistically significant difference both in the case group and the control group (P<0.01 and P=0.02 respectively). The difference in bacterial load was found to be statistically significant at time t_1 (P=0.04) between the case and control.

Discussion

This study was designed to evaluate *in vivo* the clinical and microbiological effectiveness of a combined periodontal treatment of oxygen-ozone therapy + MPT compared to conventional MPT in the treatment of subjects suffering from chronic PD. The simple biological basis for this clinical application lies in the fact that anaerobic bacteria are considered the microbes responsible for PD: a reduction in their levels or their possible eradication could lead to an improvement in the periodontal status of the subject treated.

Overall, the results indicate that the bleeding index, probing depth and microbial load of the periodontal pathogens decreased significantly

Table 1. Descriptive statistics of the clinical indices measured at different times in the experiment.

Variable	Time	Case group	Control group
PD (mm±SD)	t_0	4.13±0.66	3.98±0.60
	t_1	3.3±0.3	3.5±0.4
	t_2	3.5±0.5	3.6±0.3
Bleeding (%±SE)	t_0	89.90±1.66	86.66±1.87
	t_1	16.52±1.77	21.87±1.45
	t_2	16.95±2.22	26.65±1.84

PD, periodontal disease; SD, standard deviation; SE, standard error.

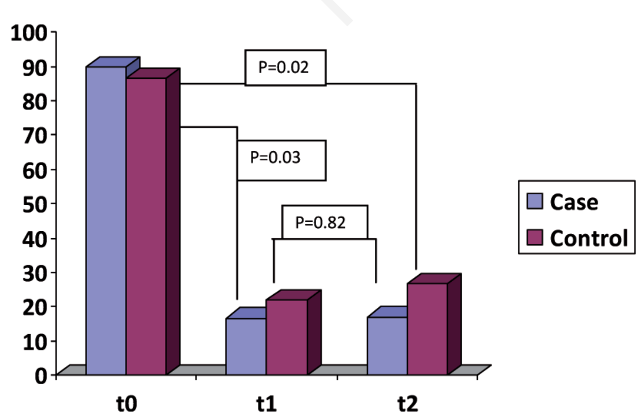

Figure 2. Bar graph of the average bleeding on probing at the three experimental times. The significance values between the two study groups (Anova analysis) are indicated. The whole model has P<0.01.

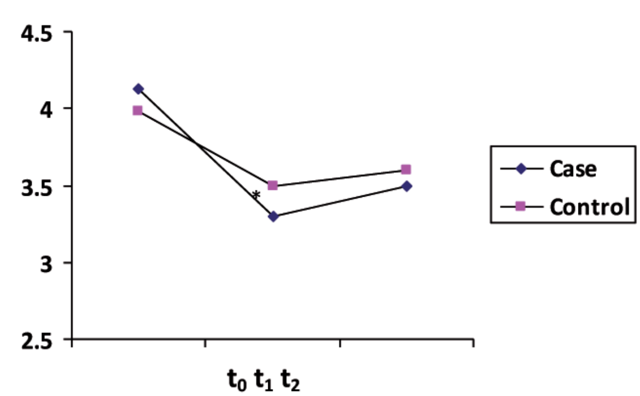

Figure 3. Line graph showing the average periodontal disease in the two study groups. *One-way Anova: P<0.01.

in both the study groups analyzed, and the values of the subjects in the group treated with mechanical therapy + oxygen-ozone therapy reduced even more markedly. This indicates that oxygen-ozone has an effect in the treatment of PD, particularly in the short term. In particular, the variation in the average BOP between the two study groups was significant both after 6-8 weeks and after 6 months, while the variation in the average PD and the variation in the total bacterial load was only significant at the first follow-up.

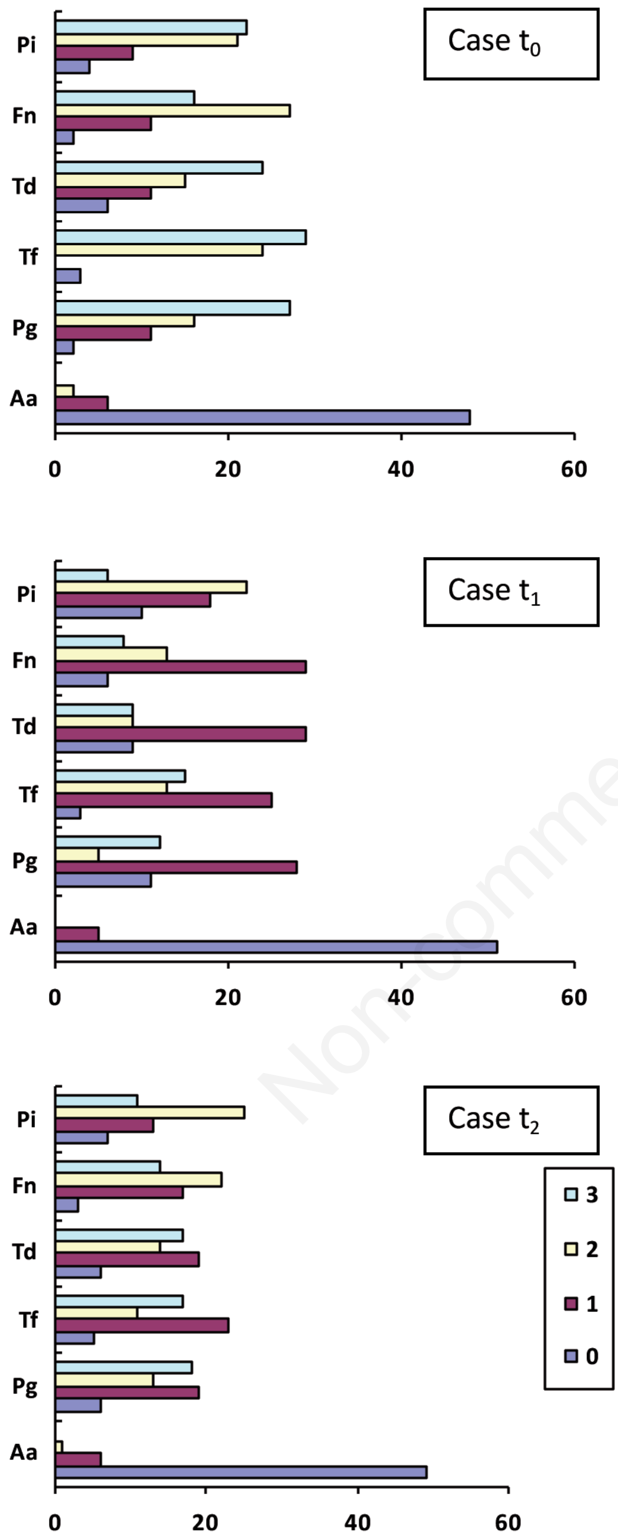

Figure 4. Bar graphs representing the bacterial load in the case group, separated into the three experimental times. 0=absent; 1=<10^5; 2=10^5; 3≥10^6.

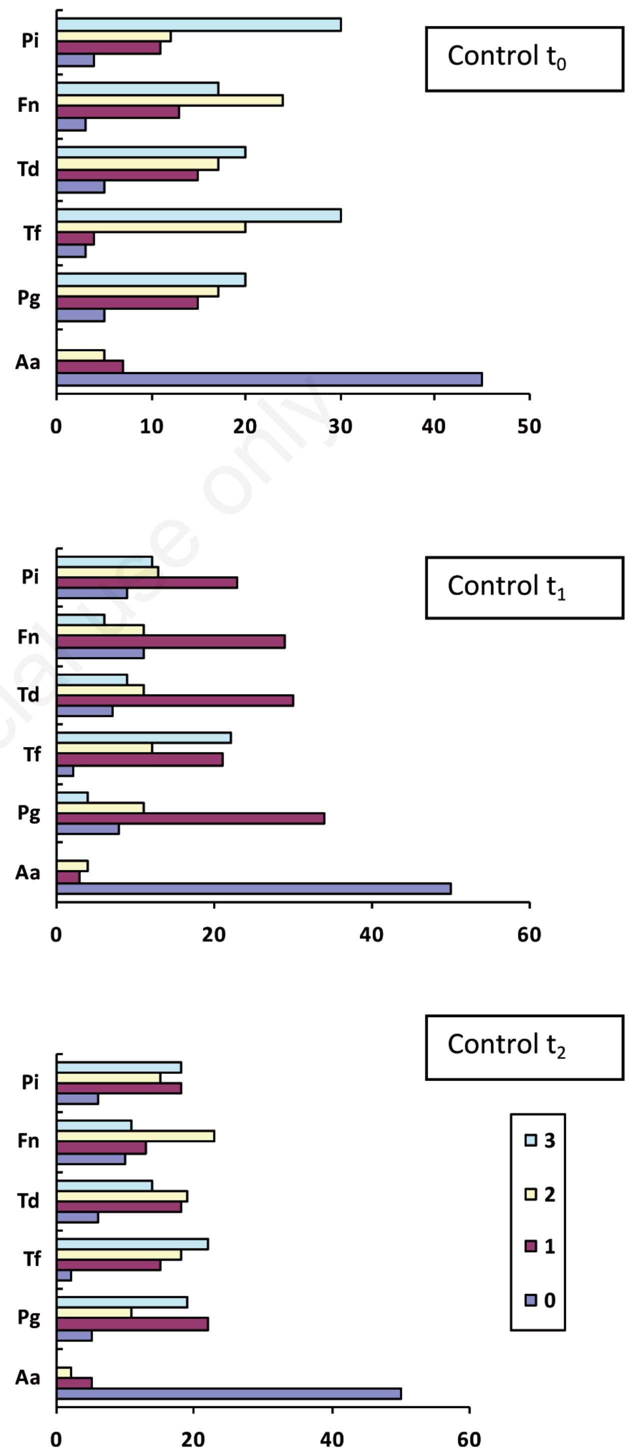

Figure 5. Bar graphs representing the bacterial load in the control group, separated into the three experimental times. 0=absent; 1=<10^5; 2=10^5; 3≥10^6.

Jiang[59] reported that, in the presence of active PD, the oxygen tension decreases inside the periodontal pockets and the amount of periodontal pathogens increases. Oxygen-ozone therapy can increase the oxygen-ozone levels locally, inhibiting anaerobic bacteria and allowing the ischemic tissues to quickly resume tissue metabolism.[43,45,49]

A study by Signoretto and associates[50] showed that MPT combined with HBO reduces the anaerobic Gram-bacteria load by up to 99.9%, and this effect lasts up to about two months after the therapy. According to Signoretto's study, HBO alone or mechanical therapy alone also have an effect on anaerobic bacteria. However, this occurs more rapidly in the group of subjects treated with HBO + mechanical therapy. HBO alone and in combination with mechanical therapy allows the value of the index of gingival bleeding to be brought to zero, and this state of gingival health persists for two months.

Similarly, studies conducted by the group of Chen[43,49] have shown the beneficial effects of HBO in the treatment of PD, effects that may last up to a year: these effects are related to the inhibition of the growth and reproduction of the subgingival plaque at the bottom of the periodontal pocket, which inhibits the initial bacterial recolonization.

However, at present there is very little data in the literature about the use of oxygen-ozone therapy at standard atmospheric pressure in the treatment of PD.

Among this data, the study by Gaggl[44] demonstrated the effectiveness in a group of patients with necrotizing ulcerative periodontitis. The study design is similar to that of this study, with the exception that 3 applications of oxygen-ozone were performed per day. The results showed that patients treated with oxygen-ozone had complete eradication or a significant reduction in the microorganisms in question (*Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*), whereas none of the subjects treated without oxygen-ozone had complete eradication of the bacteria. However, in Gaggl's study[44] the treatment protocol involved the use of antibiotics (amoxycillin with clavulanic acid, metronidazole) as well as hydrogen peroxide rinses. This approach, used in the treatment of necrotizing ulcerative periodontitis, allows the removal of periodontal pathogens localized in other ecological niches of the oral cavity, such as the tonsils or the back of the tongue, thereby delaying subgingival recolonization. In this study, it was decided not to use antibiotic treatment since systemic antibiotic therapy combined with instrumental therapy provides no clinical or microbiological benefit in chronic periodontitis.

Similarly, Schlagenhauf *et al*.[60] had good results in a study of a total of 14 patients. The research group performed irrigations with gaseous oxygen-ozone once a week for a total of 8 weeks. The study results have shown significant clinical and microbiological improvements in the group treated with oxygen-ozone.

One limitation of our study was the lack of specific information in the literature about the time and the dose of application of oxygen-ozone, which varies depending on the author.

From a purely clinical point of view, the treatment results led to a reduction in bleeding on probing (below 30%) and to a reduction in the probing depth. The clinical changes, characterized by an initial improvement followed by a period of substantial stability, are similar to those reported in other studies.[4,6,13] The average PD at the different time intervals apparently does not tend to change very much; this can be explained by the fact that the majority of sites still have small probing depths, even in the presence of periodontal disease. These sites do not show significant changes in their measurements on subsequent follow-ups, and therefore the average PD also does not change significantly. In contrast, the sites of medium and great depth were those that showed significant changes: this result was also observed in several other studies.[19,61] Clinical changes in the present investigation are accompanied by specific changes in the levels of subgingival microbial flora: overall, there was a significant reduction in periodontal bacteria both of the red complex and the orange complex in both the study groups, statistically significant at the first follow-up. In both the study groups, the different treatments were able to cause a decrease in the microbial load and only rarely was there complete eradication of the bacteria. Reduction is not predictable by the periodontist, since it is highly variable from patient to patient. Haffajee and colleagues[61] and Cugini and colleagues[19] have pointed out a significant reduction in the levels of *P. gingivalis*, *T. denticola* and *T. forsythia* and a concomitant increase in *Actinomyces spp*, *Capnocytophaga spp*, *F. nucleatum*, *Streptococcus mitis*, *Veillonella parvula*. Doungudomdacha *et al*.[62] observed no eradication of the periodontal pathogenic species, but only a reduction in the number of *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomintans*. Darby *et al*. (2001)[63] had a significant reduction in *P. intermedia*, *T. denticola* and *T. forsythia*, with little or no change in the rest of the microflora. In other cases there was complete elimination of *P. gingivalis*, with persistence of *A. actinomycetemcomitans*[64] or the persistence of *P. gingivalis*, *P. intermedia*.[65,66]

MPT can be performed successfully in 68% of patients, while in 32% of cases the therapy is able to obtain only few benefits and these patients continue to harbor high levels of putative periodontal pathogens with consequent progressive loss of attachment.[61] The limited effect of hand instruments may be due to bacterial localization in the altered cement or root dentinal tubules, in hard subgingival deposits, in furcations or other anatomical alterations, in the oral mucosa, back of the tongue, tonsils and other oral sites considered sources of microbial supply. In particular the localization has been demonstrated in the subepithelial gingival tissues of *A. actinomycetemcomitans*, in the crevicular epithelial cells by *A. actinomycetemcomitans*, Peptostreptococcus *micros*, *P. gingivalis*, *P. intermedia* and in collagenous substrate of *P. gingivalis*.[66] The incomplete elimination of periodontal pathogens by non-surgical therapy can lead to a relatively rapid recolonization and recurrence of the disease.[67-69] In the light of these considerations, it may be justified to use a local antimicrobial agent, *i.e.* oxygen-ozone, although an essential prerequisite is preliminary treatment with conventional MPT, which allows the number of bacteria within the periodontal pockets to be markedly reduced and also acts on the bacterial biofilms facilitating the action of the oxygen-ozone.

Conclusions

In conclusion, in the light of the results obtained, it can be said that, in the short term, treatment with oxygen-ozone can lead to an improvement in clinical and microbiological conditions. However, according to what is currently known, mechanical periodontal therapy is still the treatment of choice. Further longitudinal studies are needed to assess any other therapeutic protocols and any limitations and indications of the use of oxygen-ozone therapy.

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