Original Article

Aquolab® ozone-therapy is an efficient adjuvant in the treatment of chronic periodontitis: A case-control study

Francesco Carinci, Annalisa Palmieri[1], Ambra Girardi[1], Francesca Cura[2], Dorina Lauritano[3]

???, School of Medicine, University of Ferrara, Ferrara, [1]???, Center of Molecular Genetics, CARISBO Foundation, Institute of Histology and General Embryology, School of Medicine, University of Bologna, Bologna, [2]Department of Surgical and Morphological Sciences, University of Insubria, Varese, [3]Department of Interdisciplinary Medicine and Surgery, University of Milan-Bicocca, Milan, Italy

ABSTRACT

Aim: The objective of this study was to compare the efficacy of supportive periodontal therapy (i.e., scaling and root planning [SRP]) alone versus Aquolab® ozone therapy used in association with SRP in the treatment of chronic periodontitis in adult patients. **Materials and Methods:** A total of 20 patients with a diagnosis of chronic periodontitis (40 localized chronic periodontitis sites) were enrolled. None of these patients have previously received any surgical or nonsurgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Two nonadjacent sites in separate quadrants were selected in each patient to verify treatment efficacy (split-mouth design). Microbial analysis was analyzed at baseline and the 7th day after treatment. SPSS program and paired simple statistic *t*-test were used to detect statistically significant differences. **Results:** There was a statistically significant reduction of *Tannerella forsythia* loading in sites treated with ozone therapy respect to those treated with SPR alone. A similar trend was obtained also for additional 5 species and for total bacterial loading (CBT). These results were obtained with a single local application of ozone therapy just after SPR and with a molecular control 7th day after treatment. **Conclusion**: Aquolab® ozone therapy in is effective in reducing the CBT in pockets of patients affected by periodontitis. It is an efficacy medical device to be used as adjuvant therapy to be added to SRP in the management of moderate to severe chronic periodontitis.

Key words: Bone loss, ozone therapy, periodontitis, root planning, scaling

INTRODUCTION

Periodontal disease is one of the prevalent illnesses in the adult population. It is characterized by a symptom triad: Tooth mobility, foetor ex ore, gingival bleeding. If left untreated the disease can lead to tooth loss. Pathogenesis of the periodontal disease is multifactorial, and bacterial have a role.[1] The main pathogens implicated in periodontal disease are anaerobic Gram-negative bacteria of which the most aggressive were identified in the "red complex" group: *Porphyromonas gingivalis* (PG), *Tannerella forsythia* (TF), and *Treponema denticola* (TD).[2] Furthermore, *Aggregatibacter actinomycetemcomitans* (AA), *Fusobacterium nucleatum* (FN), *Campylobacter rectus* (CR) have a prominent role as well as the total bacterial loading (CBT).

The aim of periodontal treatment is to eliminate the oral infection, and prevent the progression of the disease.[3] Many studies have demonstrated that the nonsurgical therapy including scaling and root planning (SRP) associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health.[4,5]

Previous data about the effectiveness of ozone against periodontal pathogenic microorganisms are controversial. One study concluded that there were no significant differences in the effectiveness

Address for correspondence:
Dr. Dorina Lauritano,
Department of Translational Medicine and Surgery, Centre of Neuroscience, University of Milan-Bicocca, Via Cadore 48, 20052 Monza, MB, Italy.
E-mail: dorina.lauritano@unimib.it

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Website: www.jofs.in
DOI: ***
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of aqueous or gaseous ozone compared with 2% chlorhexidine.[6] Another study showed that ozone has an antimicrobial effect equivalent to that of the Er:YAG laser.[7] Furthermore, irrigation with ozonized water as an adjunctive therapy to SRP produces no statistically significant benefit compared with SRP plus distilled water irrigation.[8] On the contrary, another study reported that ozone might be considered as an alternative management strategy for periodontitis treatment due to its powerful ability to inactivate microorganisms.[9]

Aim to the present study is to evaluate the effectiveness of ozone therapy (i.e., Aquolab®, EB2C, Milan, Italy) used in association with SRP in chronic periodontitis treatment in adult patients.

Ozone therapy

Ozone (O_3) is a triatomic molecule consisting of three oxygen atoms and presents many applications in medicine and dentistry. O_3 can decrease bacterial, fungi, virus and protozoa loading. Its effects are antimicrobial, analgesic and bio-stimulants. Ozone allows a better oxygenation of tissues and promotes healing, increases cell energy by stimulating the Krebs cycle production of adenosine triphosphate, stimulates white blood cells and the immune system response production of cytokines, increases blood flow and the elasticity of erythrocytes.

Aquolab® ozone therapy is minimally invasive and at the level of the oral cavity, has no side effects [Figures 1 and 2].[10,11]

MATERIALS AND METHODS

In the period, September to October 2014, 20 patients with a diagnosis of chronic periodontitis were randomly selected. Patients were enrolled if they have two sites located in separate quadrants which required periodontal treatment, in the age group of 35-55. Subjects have not received previously any surgical or nonsurgical periodontal therapy. The patients were excluded from the study if they meet any of the following criteria:

1. Pregnancy;
2. A history of taking antibiotics or using antibacterial mouth rinses for past 6 months;
3. Teeth with furcation involvement;
4. Smoking, drug or alcohol abuse.

Subjects participating in the study volunteered will follow a detailed verbal description of the procedure and by signing consent forms.

Clinical methods

A total of 20 patients (i.e., 40 sites) were selected and grouped into two categories: Control and test (split-mouth design). The control group (20 sites) was treated with SRP without using Aquolab® ozone therapy (control site). The test group (20 sites) was treated by SRP plus Aquolab® ozone therapy (test site).

All patients underwent to SRP at the baseline measurement. Prior to SRP microbial analysis was performed in each selected site. Then SRP was done at both sites using manual scalers. After SRP, Aquolab® ozone therapy (EB2C, Milan, Italy) was adjunct in the test site of each enrolled patient [Figure 3]. The treatment was delivered for 30 s with a concentration of dissolved ozone between 0.01 and 0.03 ppm (average 0.02 ppm). After 1-week, microbiological samples were collected again from both sites in each patient.

Aquolab® ozone therapy did not have any side effects or discomfort or adverse reactions, nor immediate and after a week control time. No patient reported pain, burning, tingling sensation or numbness. The Aquolab® ozone therapy was also tasteless and odorless.

Figure 1: Design of instrumentation used to deliver ozone to the oral cavity

Figure 2: Photo of the display

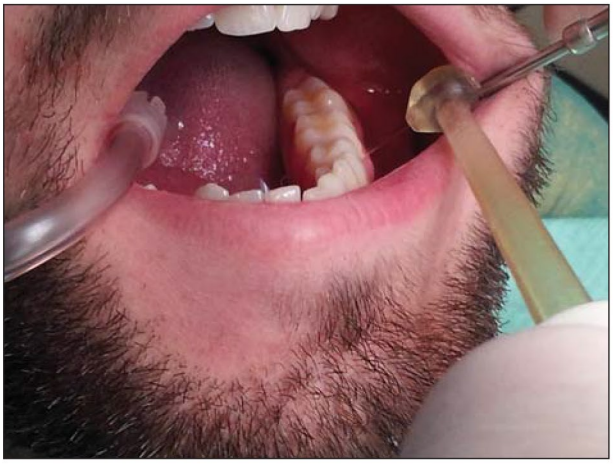

Figure 3: Ozone therapy delivered in periodontal pocket

For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgingival samples and were immediately transferred to the microbiological laboratory for processing. AA PG, TF, TD FN, CR, and CBT were evaluated.

Real-time polymerase chain reaction

Probes oligonucleotides were designed basing on 16S ribosomal RNA (rRNA) gene sequences of the Human Oral Microbiome Database (16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequence or less conservate spots. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conserved sequence of the 16S rRNA gene. The second reaction detected and quantified all selected bacteria in two multiplex PCR. This reaction included a total of twelve primers and six probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Bio systems 7500 Sequence Detection System. The amplification profile were initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments were performed including no template controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as standard for the quantitative analysis. Standard curves for each target were constructed in two triplex reactions, using a mix of the same amount of plasmids, in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions (data not shown). The copy numbers of individual plasmid preparations was estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of the relative amount of red complex species. To prevent samples and PCR contamination, plasmid purification and handling was performed in a separate laboratory with dedicated pipettes.

Statistical analysis

SPSS program and paired simple statistic *t*-test were used to detect statistical significant differences.

RESULTS

Tables 1 and 2 report results obtained in 560 reactions. The suffix C indicates the control side whereas S the treated side. Zero (i.e., 0) is the pretreatment assessment whereas 1 is the control performed 1-week after a single application. Therefore in the case of the evaluation of single bacteria (such as AA) will have four possibilities: AAC0-bacterial loading of pretreatment control site, AASO-bacterial loading of pretreatment test site, AAC1-bacterial loading aftercare control site and AAS1-bacterial loading after Aquolab® ozone therapy.

Tables reported the following evaluations:

1. AACO versus AAC1-verification of standard therapy without Aquolab®;
2. AASO versus AAS1-checking effectiveness standard therapy plus Aquolab®;
3. AACO versus AASO-verification if there is any statistically significant difference between test and control side before any therapy Aquolab® (which could distort the final result);
4. AAC1 versus the AAS1-checking impact of adjunct of Aquolab® to standard treatment.

Only TFS0-TFS1 (i.e., comparison between pre- and posttreatment loading values of TF) reached statistical significance ($P = 0.021$) thus demonstrating that a single application of Aquolab® is effective to reduce the loading of TF in periodontal pockets.

Even if all the remaining comparisons did not reach the statistical significance, in all interaction between controls versus test site there was always a better *P* values in test site, which mean that Aquolab® has a detectable positive effect on bacteria. In fact, in Table 2, *P* values of control site (C) before and after treatment-

Table 1: Main values of each single bacterial type

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	AAC0	14,9000	20	66,6348	14,9000
	AAS0	39,7000	20	123,5374	27,6238
Pair 2	CRC0	44772,95	20	142177,7	31791,89
	CRS0	67432,10	20	189639,1	42404,58
Pair 3	CTC0	1189296	20	2126443	475487,2
	CTS0	1128709	20	2539253	567794,3
Pair 4	FNC0	276198,2	20	802547,2	179455,0
	FNS0	217724,4	20	479741,7	107273,5
Pair 5	PGC0	8653,5000	20	35197,30	7870,3560
	PGS0	81624,75	20	269014,4	60153,45
Pair 6	TDC0	22685,60	20	58767,44	13140,80
	TDS0	39178,35	20	88709,15	19835,97
Pair 7	TFC0	19686,40	20	51882,00	11601,17
	TFS0	18498,60	20	33505,97	7492,1619
Pair 8	AAC0	14,9000	20	66,6348	14,9000
	AAC1	65,8500	20	294,4902	65,8500
Pair 9	AAS0	39,7000	20	123,5374	27,6238
	AAS1	,0000	20	,0000	,0000
Pair 10	CRC0	44772,95	20	142177,7	31791,89
	CRC1	6499,4000	20	20083,84	4490,8829
Pair 11	CRS0	67432,10	20	189639,1	42404,58
	CRS1	13379,55	20	41694,32	9323,1340
Pair 12	CTC0	1189296	20	2126443	475487,2
	CTC1	872274,3	20	1399451	312926,7
Pair 13	CTS0	1128709	20	2539253	567794,3
	CTS1	395144,6	20	647981,5	144893,1
Pair 14	FNC0	276198,2	20	802547,2	179455,0
	FNC1	31545,85	20	60537,68	13536,64
Pair 15	FNS0	217724,4	20	479741,7	107273,5
	FNS1	25525,10	20	43813,86	9797,0768
Pair 16	PGC0	8653,5000	20	35197,30	7870,3560
	PGC1	40461,05	20	156634,3	35024,50
Pair 17	PGS0	81624,75	20	269014,4	60153,45
	PGS1	1269,1500	20	4166,8008	931,7250
Pair 18	TDC0	22685,60	20	58767,44	13140,80
	TDC1	9966,9000	20	28687,19	6414,6507
Pair 19	TDS0	39178,35	20	88709,15	19835,97
	TDS1	2855,9500	20	7552,7655	1688,8497
Pair 20	TFC0	19686,40	20	51882,00	11601,17
	TFC1	4896,4000	20	15815,61	3536,4773
Pair 21	TFS0	18498,60	20	33505,97	7492,1619
	TFS1	2670,8000	20	8152,1412	1822,8742
Pair 22	AAC1	65,8500	20	294,4902	65,8500
	AAS1	,0000	20	,0000	,0000
Pair 23	CRC1	6499,4000	20	20083,84	4490,8829
	CRS1	13379,55	20	41694,32	9323,1340
Pair 24	CTC1	872274,3	20	1399451	312926,7
	CTS1	395144,6	20	647981,5	144893,1
Pair 25	FNC1	31545,85	20	60537,68	13536,64
	FNS1	25525,10	20	43813,86	9797,0768
Pair 26	PGC1	40461,05	20	156634,3	35024,50
	PGS1	1269,1500	20	4166,8008	931,7250
Pair 27	TDC1	9966,9000	20	28687,19	6414,6507
	TDS1	2855,9500	20	7552,7655	1688,8497
Pair 28	TFC1	4896,4000	20	15815,61	3536,4773
	TFS1	2670,8000	20	8152,1412	1822,8742

that is Pair 8 (0.330), Pair 10 (0.254), Pair 12 (0.408), Pair 14 (0.195), Pair 16 (0.390), Pair 18 (0.407) and Pair 20 (0.240)-are always higher than the test site (S)-Pair 9 (0.167), Pair 11 (0.234), Pair 13 (0.211), Pair 15 (0.092), Pair 17 (0.195), Pair 19 (0.078) and Pair 21 (0.021), respectively.

In Table 2, Pair 1-7 are not statistically significant that means that there was the difference in the test and control site before treatment. Pair 22-28 demonstrated that there was also no difference between test and control site after treatment, which means that average bacterial loading in the periodontal pocket was homogeneous at the end of the observation period.

Table 2: Paired *t*-test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	AAC0 - AAS0	-24,8000	82,0799	18,3536	-63,2146	13,6146	-1,351	19	,192
Pair 2	CRC0 - CRS0	-22659,2	244341,4	54636,40	-137014	91696,16	-,415	19	,683
Pair 3	CTC0 - CTS0	60587,45	3287017	734999,3	-1477784	1598959	,082	19	,935
Pair 4	FNC0 - FNS0	58473,80	948634,1	212121,0	-385501	502448,2	,276	19	,786
Pair 5	PGC0 - PGS0	-72971,3	273532,5	61163,73	-200988	55045,90	-1,193	19	,248
Pair 6	TDC0 - TDS0	-16492,8	108953,7	24362,80	-67484,7	34499,17	-,677	19	,507
Pair 7	TFC0 - TFS0	1187,8000	45613,67	10199,53	-20160,1	22535,66	,116	19	,909
Pair 8	AAC0 - AAC1	-50,9500	227,8553	50,9500	-157,5896	55,6896	-1,000	19	,330
Pair 9	AAS0 - AAS1	39,7000	123,5374	27,6238	-18,1173	97,5173	1,437	19	,167
Pair 10	CRC0 - CRC1	38273,55	145506,4	32536,22	-29825,5	106372,6	1,176	19	,254
Pair 11	CRS0 - CRS1	54052,55	196467,0	43931,35	-37896,8	146001,9	1,230	19	,234
Pair 12	CTC0 - CTC1	317021,8	1673844	374282,9	-466361	1100405	,847	19	,408
Pair 13	CTS0 - CTS1	733564,1	2535576	566972,1	-453122	1920250	1,294	19	,211
Pair 14	FNC0 - FNC1	244652,4	814837,6	182203,2	-136703	626008,1	1,343	19	,195
Pair 15	FNS0 - FNS1	192199,3	484858,6	108417,7	-34721,5	419120,1	1,773	19	,092
Pair 16	PGC0 - PGC1	-31807,6	161640,9	36144,00	-107458	43842,72	-,880	19	,390
Pair 17	PGS0 - PGS1	80355,60	267418,9	59796,69	-44800,3	205511,5	1,344	19	,195
Pair 18	TDC0 - TDC1	12718,70	67147,34	15014,60	-18707,2	44144,62	,847	19	,407
Pair 19	TDS0 - TDS1	36322,40	87188,71	19495,99	-4483,17	77127,97	1,863	19	,078
Pair 20	TFC0 - TFC1	14790,00	55546,97	12420,68	-11206,8	40786,78	1,191	19	,248
Pair 21	TFS0 - TFS1	15827,80	28198,71	6305,4231	2630,3978	29025,20	2,510	19	,021
Pair 22	AAC1 - AAS1	65,8500	294,4902	65,8500	-71,9756	203,6756	1,000	19	,330
Pair 23	CRC1 - CRS1	-6880,15	44539,64	9959,3658	-27725,3	13965,04	-,691	19	,498
Pair 24	CTC1 - CTS1	477129,7	1522298	340396,1	-235327	1189587	1,402	19	,177
Pair 25	FNC1 - FNS1	6020,7500	75512,39	16885,08	-29320,1	41361,64	,357	19	,725
Pair 26	PGC1 - PGS1	39191,90	155192,3	34702,04	-33440,3	111824,1	1,129	19	,273
Pair 27	TDC1 - TDS1	7110,9500	27384,98	6123,4681	-5705,62	19927,52	1,161	19	,260
Pair 28	TFC1 - TFS1	2225,6000	17654,75	3947,7225	-6037,08	10488,28	,564	19	,580

DISCUSSION

In our study only, TFS0-TFS1 reached statistical significance thus showing that Aquolab® is effective in treating at least one bacterial agent involved in periodontal disease. In addition, all *P* values detected in test site were lower than those detected in the control site thus showing a general trend for all bacterial species. The fact that only TFS0-TFS1 reached statistical significance is probably related to the single application. If more applications will be delivered (for example one a day as it is for toothbrush), a greater reduction in bacterial loading could be detected. It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic microorganisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health. Mechanical debridement is effective in both disturbing the biofilm and reducing the bacterial load. However, sometimes-mechanical instrumentation may not be sufficient to control the disease due to tissue invasive pathogens or other tooth-related anatomic factors. In such conditions, adjunctive use of Aquolab® ozone therapy can provide an additional benefit in controlling the disease.

Support periodontal therapy is widely used, but a better effectiveness is demonstrated by the administration of ozone therapy in the association. The advantages of Aquolab® ozone therapy involve the use of ozone directly into the periodontal pocket, minimizing the adverse

effects related to systemic therapy.[5] The potential benefits of Aquolab® ozone therapy include improved patient compliance and easier access to a periodontal pocket. Aquolab® ozone therapy devices have been used either alone or as adjunct with SRP. Aquolab® ozone therapy is administered directly into the periodontal pocket, and the effectiveness of this local therapy is related to their bactericidal activity and the subsequent reduction of gingival inflammation.

In contrast with adjuvant traditional periodontitis treatments, such as antibiotics and chemical devices, Aquolab® ozone therapy is quite chip. The Aquolab® ozone therapy is minimally invasive and has demonstrated to reduce bacterial loading.

The topical use of Aquolab® ozone therapy along with mechanotherapy improves clinical results, and at the same time is free from adverse effects. Local ozone therapy into the pocket achieves a greater reduction of bacterial loading, proving bactericidal for most periopathogens.

Within the limitations of the current study, due to limited sample, we evaluated the efficacy of ozone therapy in the management of moderate to severe chronic periodontitis. The results of this investigation demonstrated an overall improvement in specific CBT.

Microbiological testing was thought appropriate to evaluate the effect of ozone therapy on subgingival microbial population, the primary etiological factor for periodontitis. Several methods have been used for microbiological testing in periodontitis.[12] It is well-known that both PG and TD occur concomitantly with the clinical signs of periodontal destruction.[13,14] They appear closely "linked" topologically in the developing biofilm, shown an *in vitro* ability to produce a number of outer membrane-associated proteinases. They are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover, both them and TF, show a higher prevalence in disease than in health suggesting that these bacterial are associated with the local development of periodontitis.[15] Our results were able to detect a statistical significant effect on TF. It is possible that higher concentration and more local application could have a wider effect on the additional component of red complex triad. Additional studies are needed to proof this last point. As previously mentioned, the limitation of the study is related to the single application. A further study involving a larger number of patients using Aquolab® everyday as a home care tool will aid to demonstrate the real efficacy of this complementary therapy.

CONCLUSION

Aquolab® ozone therapy is an effective adjuvant therapy which should be added to SRP in the management of moderate to severe chronic periodontitis.

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How to cite this article: We will update details while making issue online***

Source of Support: Nil, **Conflict of Interest:** None declared