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A topical desiccant agent in association with ultrasonic debridement in the initial treatment of chronic periodontitis: a clinical and microbiological study

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SUMMARY

Effective sub-gingival debridement is crucial to prevent serious systemic infections in hospitalized patients. Lack of compliance and the impracticality of repeated treatment in a short span of time are identified barriers to the performance of full mouth scaling and root planing (SRP). The aim of this randomized study was to evaluate the clinical and microbiological effects of the adjunctive administration of a locally delivered desiccant liquid with molecular hygroscopic properties (HYBENX® Oral Tissue Decontaminant™; HBX) in association with sub-gingival ultrasonic debridement (UD) in a hospital setting. Sixteen patients presenting moderate to severe chronic periodontitis were followed in a randomized 3 month, split-mouth, single-blind, prospective study. At baseline (T1) control and test sides were treated with supra and subgingival UD with or without the association of a locally delivered desiccant liquid (HBX). Treatment was repeated after 6 weeks (T2). Clinical and microbiological parameters were assessed at T1, T2 and at 3 months (T3). The test group sites presented a significantly greater reduction in visible plaque index (VPI), bleeding on probing scores (BOP) and gingival index (GI) at T2 and T3 compared to the control group sites.

HBX as monotherapy reached the same bacterial load reduction as UD. Compared to UD, a combined HBX-UD treatment resulted in a statistically significant greater bacterial load reduction immediately after treatment. A significantly lower anaerobic bacterial load was still present at T2. Data obtained show that decreased inflammatory signs and reduction of the bacterial load can be obtained in the short term by topical association of the desiccant agent HBX with UD.

KEY WORDS: Periodontitis, Disinfection, Anaerobic bacterial load, Ultrasonic debridement, Topical agent.

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INTRODUCTION

The presence of bacterial plaque, structured as biofilm, represents the main etiological factor involved in the initiation and progression of periodontitis (Offenbacher, 1996). Effective preventive periodontal therapy is centered on anti-infective procedures aimed at reducing or even eradicating pathogenic organisms found in dental plaque-associated biofilm.

Manual scaling and root planing (SRP) performed in sextant, quadrant or in full mouth manner has been traditionally considered the treatment of choice to obtain meticulous subgingival debridement, and it has been proven to be effective in improving gingival health conditions and in reducing periodontal inflammation (Adriaens and Adriaens, 2004, Badersten *et al.*, 1984, Cobb, 1996, Kaldahl *et al.*, 1996, Pihlstrom

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et al., 1983, Ramfjord *et al.,* 1987, Suvan, 2005). However this procedure is time-consuming and exhausting for the patient, and may not be suitable in a hospital setting. In the last decade a full mouth ultrasonic debridement procedure (FMUD) was shown to be at least as effective as the quadrant SRP, but easier to perform and better tolerated by patients (Smart *et al.,* 1990, Tunkel *et al.,* 2002, Walmsley *et al.,* 2008). On this basis, FMUD has been widely adopted among physicians, especially for ergonomic reasons.

However, regardless of the subgingival instrumentation method applied, in some patients the complete resolution of certain inflammatory lesion sites might not be achieved, either by manual or mechanical procedures, forcing clinicians to repeated treatment sessions in a short span of time to eradicate infection (Greenstein, 2002; Serino *et al.,* 2001; Tomasi *et al.,* 2006; Tonetti *et al.,* 1998).

In a hospital setting where patients often present physical impairments and/or limiting conditions requiring specialized services or integrated health care programs, it is much more appropriate to provide the painless and the shortest single treatment sessions with the highest antibacterial efficacy as possible.

In recent years, the adjunctive topical administration of antimicrobials has been proposed to improve subgingival debridement efficacy. Adjunctive use of controlled-release, locally delivered antibiotic agents has been shown to significantly improve the treatment outcomes of periodontal debridement especially in deep pockets (Hallmon and Rees, 2003, Hanes and Purvis, 2003, Lang *et al.,* 2008, Quirynen *et al.,* 2002, Wennstrom *et al.,* 2001). However, in case of relapse of disease, the concern about the risk for the emergence of resistant bacterial strains dictated prudent administration and limited repeated administrations have been recommended (Herrera *et al.,* 2008, Walker *et al.,* 2000).

When the use of antiseptic agents has been suggested to improve UD efficacy, favorable but sometimes contradictory and low magnitude results have been found (Del Peloso Ribeiro *et al.,* 2006, Koshy *et al.,* 2005, Leonhardt *et al.,* 2007, Rosling *et al.,* 2001).

Various factors can severely hamper the effectiveness of subgingival instrumentation and local pharmacological therapeutic action (D'Aiuto *et al.,* 2005, Tomasi *et al.,* 2007) but the main mechanism of bacterial protection remains their organization into a biofilm (Socransky and Haffajee, 2002).

In biofilms microorganisms live in a self-produced hydrated biomatrix constituted for 10-30% by extracellular polymeric substances and for 70% by water, which hinders mechanical attempts at complete biofilm removal during basic therapy and prevents antimicrobial agents from reaching the intended bacterial targets in the subgingival area (Marsh, 2005, Stoodley, *et al.,* 2002). On the basis of its porous structure and high water content, it can be expected this bio-matrix might lose its integrity once exposed to the topical action of a strong desiccant agent with hygroscopic properties and subgingival biofilm became particularly vulnerable to mechanical removal procedures.

Recently, data have been provided in human on the safety and effectiveness of a new product with strong molecular desiccation properties introduced for topical use in the treatment of oral aphtae (Porter *et al.,* 2009). This product is a simple liquid solution of sulfonated phenolics, which has been shown to possess strong contact desiccant properties: when placed onto susceptible organic material the mixture instantly absorbs free and electrostatically-bonded water and denatures the molecular structure of the organic attachment matter.

To the best of our knowledge, there was no prior controlled clinical study in the literature evaluating the safety and the possible beneficial effects of the preventive topical administration of a desiccant agent as an adjunct to subgingival instrumentation in the treatment approach for chronic periodontitis.

Hence, the aim of the present prospective, single blind, split-mouth study was to assess the safety and the efficacy of an oral biofilm-disrupting agent (HYBENX® Oral Tissue Decontaminant™; HBX) as an adjunct to improve the supra and subgingival antibacterial UD efficacy.

MATERIALS AND METHOD

Study design

The study was designed as a 3-month, split mouth, randomized, prospective, controlled,

single-masked study to compare the clinical and microbiological outcomes of the full-mouth ultrasonic debridement procedure in association with the supra and subgingival topical administration of an oral tissue decontaminant liquid with hygroscopic properties in the initial treatment approach to chronic periodontitis (Figure 1). The experimental protocol was reviewed and approved by the Ethics Committee of the University of Verona (Prot. HX-GL-ITA1. Date of approval: 2013-20-11), and all patients were informed about the nature of the proposed treatment and signed informed consent.

Study population

All patients involved in this study were recruited from the pool of periodontal patients of the Clinic of Dentistry and Maxillo-Facial Surgery, University of Verona, Italy. Twenty subjects were originally recruited according to the protocol. Examinations in the present study were carried out from January 2014 to June 2014.

Inclusion criteria

- diagnosis of mild or severe chronic periodontitis by the presence of periodontal pockets with a clinical attachment loss ≥5 mm, bleeding on probing (BoP) and radiographic bone loss (Armitage, 1999, Flemmig, 1999);
- a minimum of 4 teeth per quadrant (wisdom teeth were not included in the examination);
- at least eight teeth with pocket depth (PPD) ≥5 mm with radiographic signs of bone loss (Qualifying sites);
- of the eight teeth, at least two teeth had to present a PPD equal or greater than 7 mm and bleeding after pocket probing and two had to present a PPD 5-6 mm and bleeding after probing;
- healthy patients according to medical history and clinical judgment.

Exclusion criteria

- allergy to sulfonated compounds;
- subgingival instrumentation within 3

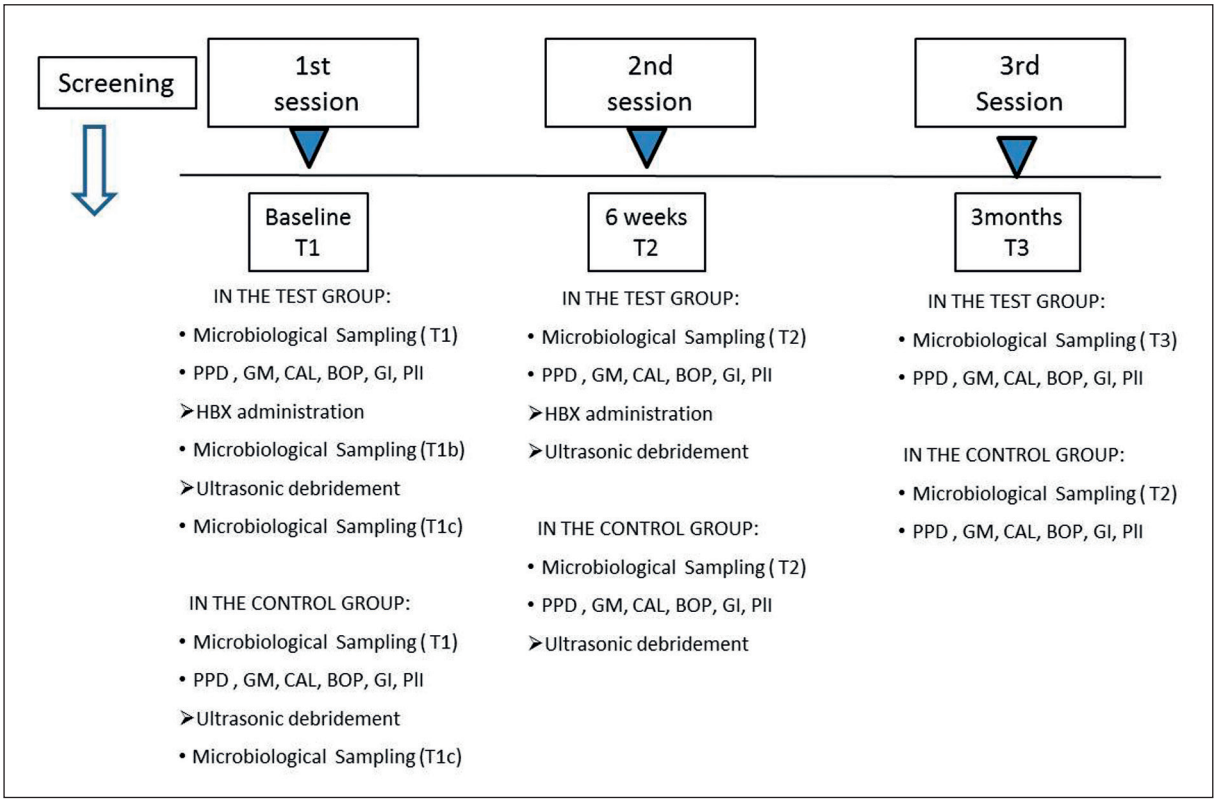

FIGURE 1 - *Flow chart of the study.*

months prior to the screening examination;
- compromised heart condition, diabetes, or any other systemic disorder that would require antibiotic prophylaxis;
- pregnancy or nursing;
- consumption of drugs that could affect the clinical features of periodontitis or the response to periodontal treatment within 3 months prior to the start of the study (antibiotic, anti-inflammatory, anticonvulsant, immunosuppressant and calcium channel blocker);
- smoking.

Clinical variables

Clinical parameters were recorded immediately before the treatment at the baseline, (T1), after 6 weeks (T2) and after 3 months (T3).

The parameters were measured at six sites on each tooth (mesiolingual, lingual, distolingual, distobuccal, buccal and mesiobuccal) using a manual standardized periodontal probe with 1mm markings *(*Bontempi Surgical Instruments, Tuttlingen, Germany). The clinical parameters evaluated were the following:

- Visible Plaque Index (VPI) (Ainamo and Bay, 1975): calculated dichotomously as the number of sites demonstrating plaque accumulation at the cervical part of the tooth by running a probe along the tooth surface;
- Bleeding on Probing (BoP) (Muhlemann and Son, 1971): calculated dichotomously as the number of sites demonstrating bleeding within 15 seconds following gentle pocket probing at six sites per tooth;
- Gingival Index (GI) (Loe and Silness, 1963): measured after gentle probing at four sites for tooth as follows: (0) no signs of inflammation, (1) signs of inflammation but not bleeding, (2) line of bleeding, (3) drop of bleeding, (4) spontaneous bleeding;
- Probing Pocket Depth (PPD): the distance from the gingival margin and the bottom of the sulcus or the pocket;
- Gingival Margin Location (GM): the distance from the cementum-enamel junction and the GM level;
- Clinical Attachment Level (CAL): the distance from the CEJ (cementum enamel junction) to the base of the sulcus or pocket.

When the CEJ or a fixed reference point (i.e. the margin of a restoration) was not clearly identifiable, a customized acrylic stent with a guiding groove served as a reference guide for measuring CAL and GM recession.

Clinical parameters were assessed and microbial samples were taken by a blinded investigator who was not involved in treatment and who was not informed on the choice of treatments provided (PA). Before the start of the study the investigator was calibrated for intra-examiner adequate levels of accuracy and reproducibility in recording the clinical parameters and indices. Three patients with chronic periodontitis were enrolled for this purpose. Duplicate measurements for PPD and CAL were collected with an interval of 24 hours between the first and second recording. The intra-class correlation coefficients, used as a measure of intra-examiner reproducibility had to be greater than 0.8 for mean PPD and CAL (Data not shown).

Test substance and administration

The tested material (HYBENX® Oral Tissue Decontaminant™, EPIEN Medical, MN USA) is a concentrated aqueous solution of sulfonated aromatics and free sulfate. It is cleared for human oral use by both the US FDA and the EU Commission and intended for use as a professional irrigation solution for topical supra and subgingival placement during standard dental procedures. HBX exhibits a strong contact desiccant action which provides enhanced debridement and cleansing of pathologic matter from tissue surfaces beyond what is achievable with current standard mechanical irrigation solutions. When it is placed onto susceptible organic material, the product instantly absorbs free and electrostatically bonded water, denaturing the molecular structure of the organic matter. Biofilm is expected to be especially sensitive to the disruptive action of HBX solution due to its porous structure and high water content.

On the test side of this study, HBX was administered before the ultrasonic treatment and left in contact with supra and subgingival plaque biofilm for up to 60 seconds, then rinsed with water and evacuated.

Therapeutic procedures

All the treatments were performed by only one dental hygienist (the operator) experienced re-

garding the various procedures. The operator recorded the time required for the instrumentation and re-instrumentation procedures.

Screening visit
At a screening visit, (which was scheduled no more than 2 weeks prior to the baseline treatment (T1), general oral and full mouth periodontal examinations including full-mouth probing and radiographic evaluation were performed. In conjunction with the screening examination, all patients were instructed on the causes and consequences of periodontal disease and how to perform proper oral hygiene. Supragingival plaque retention factors were removed, and cavities were filled.

Stratification and randomization procedures and initial treatment (T1)
Two weeks after the screening visit all subjects were recalled and received the baseline examination and the first treatment session (T1). After the baseline examination and immediately before the treatment procedure, the therapy methods (Test or Control) were randomly allocated to one of the patient's sides using a predetermined computer-generated randomization scheme.
After the randomization procedures, in the test side, immediately before the ultrasonic instrumentation all sites received a supragingival administration of the test substance. All sites showing a PPD ≥5 mm at T1 (Qualifying sites) received an adjunctive subgingival 45-60 second administration of the product. This was slowly expressed by the use of the delivery syringe into the periodontal pocket, starting from the base of the pocket, until it reached the gingival margin; it was left in place to act for 45-60 seconds, and rinsed away by abundant irrigation with saline solution. Given the extensive continuous irrigation provided during UD, no placebo was used in control side. After HBX administration, all sites independent of the randomized assignment, were subjected to a maximum 45 minutes single episode of full-mouth, supra- and sub-gingival ultrasonic instrumentation using a piezoceramic ultrasonic scaler (Piezon Master 400, EMS, Nyon, Switzerland) equipped with standard tips, water coolant, and power setting of 75% without any adjunctive pre- or post-treatment. The criterion for a thorough subgingival debridement was a smooth root surface free of bacterial plaque and calculus verified by magnifying lenses. No local anesthesia was administered during debridement. All participants were advised not to use antiseptic mouthwash during the course of treatment, so that plaque control could be achieved solely by optimal tooth brushing.

Six-week re-evaluation and repeated treatment (T2)
After 6 weeks (T2) the patients were scheduled for re-evaluation, and all qualifying sites (i.e., sites showing PPD ≥5 mm at baseline) underwent subgingival ultrasonic instrumentation and HBX administration following the baseline scheduled treatment protocol, independently from the BoP status.

Three-month (T3) reevaluation
At three months (T3) clinical final re-evaluation was performed. At any interval examinations treatment allocation was concealed to the examiner.

Treatment time evaluation
At each treatment session the operator recorded separately the time required for the ultrasonic instrumentation for each treatment side; in the test side he was also asked to record separately the time required for the topical administration of the test substance.

Patient's treatment perception
Patient's treatment perception was recorded at the end of the first treatment session using a visual analogue scale (VAS). Zero value indicates the absence of pain and 100 an unbearable pain. Immediately after treatment all patients were asked to mark the level of pain they experienced in the test and control side treatment.
As a parameter for the patient's discomfort, they were also asked to record if they experienced subjective postoperative hypersensitivity during the first week after the treatment session.

Microbiological examination
At baseline, after random assignment, subgingival plaque samples (approximately 1 mg) were

collected from each patient before treatment (T1a) and immediately after treatment (T1b). The procedures were repeated before treatment, after 6 weeks (T2) and after 3 months (T3). To assess whether the topical agent has some effectiveness even if administered alone, in the test group sites the sampling procedure was also repeated immediately after the topical administration (T1b). Samples were collected using a sterile periodontal 3/4 Gracey curette at least from two mesiobuccal or distobuccal qualifying sites, possibly comparable for PPD, position and tooth type, in both control and test sides of the mouth.

After careful supra-gingival plaque removal the tooth was isolated with cotton rolls and the curette inserted to the bottom of the pocket and moved coronally in contact with the root surface with a single scaling stroke to remove the most apical plaque. Each plaque sample was immediately suspended in a sterile tube containing 1 ml of thioglycollate medium (BD Difco) kept in melting ice, and transferred under anaerobic conditions without shaking to the laboratory for microbiological evaluation. The maximum time between sample collection and laboratory processing was 1 hour. The evaluation of the microbial samples was blind. Before microbiological procedures, plaque samples were thoroughly shaken for 30 s in a Vortex mixer and exposed for 30 s to an ultrasonic bath (Branson mod. 1210). This treatment was the minimum needed to obtain the highest disaggregation and dispersion of bacteria in the sample without interference with culturability (data not shown). SuiTab. dilutions (10 fold) of each plaque sample were plated on Columbia blood agar (5% sheep blood, BD Difco) to evaluate aerobic and facultative bacteria counts and Schaedler K-V Agar containing 5% Sheep Blood, kanamycin and vancomycin (BD Difco) to evaluate cell counts of strict anaerobic bacteria. Plates were incubated at 37°C for 48 hours: Columbia blood agar plates in an atmosphere enriched with 5% CO_2 as previously described (Signoretto, *et al.,* 2013) while Schaedler blood plates were placed in an anaerobic chamber (Whitley DG 250 Anaerobic Workstation, Don Whitley Scientific, Shipley, UK) with an atmosphere composed of 85% nitrogen, 10% hydrogen and 5% CO2 (Signoretto, *et al.,* 2014). Resulting colonies were counted and numbers reported per mg of plaque sampled.

Data analysis

The site was regarded as the evaluation unit**.** The primary outcome parameter was the change in BoP. Due to the absence of previous data to base the sample size calculation, the number of patients was chosen based on similar studies (Christgau, *et al.,* 2007).

The secondary outcome parameters were the changes in VPI score, GI value, and bacterial UFC/ml count differences as well as the occurrence of root hypersensitivity between methods.

The distribution of continuous variables was analyzed initially with the Shapiro-Wilk W test, and data, due to their deviation from the Gaussian distribution, are reported as median interquartile range (IQR). To allow comparison with other published studies, mean values and standard deviation are also reported.

Comparisons in microbiologic data were made between the aerobic and anaerobic total cultivable counts at baseline, immediately before (T1a) and immediately after the completion of the retreatment (T1b), and after 6 weeks (T2) and 3 months (T3) from the baseline. The statistical analysis was performed on log-transformed data.

Differences between treatment group median values were assessed by Wilcoxon rank-sum test.

Treatment time is reported as mean values and standard deviation. The comparison between mean time was performed using Student's t-test for paired data.

Differences between frequencies were assessed by chi square test, with Yates correction for continuity.

All statistical tests were two-tailed and conducted at a significance level of $p<0.05$. The analysis was performed by using Stata/SE 10 (College Station - USA).

RESULTS

Four patients did not show up for all the appointments because of failure to comply with the scheduled appointments for reasons not re-

lated to the study; thus, a total of 16 patients completed the 3 month study. Table 1 illustrates the study population demographic characteristics.

Clinical result

At baseline no significant dissimilarities in qualifying sites distribution and in the moderate and deep pockets prevalence (Table 2) or clinical differences (Table 3) between group sites were found. Clinical results are strictly referred to qualifying sites (Table 4).

In this study, when differences with baseline were compared between group sites the test treatment caused both at T2 and at T3 significantly greater VPI, BoP, GI reductions than the control treatment, but failed to provide significant differences in PPD, GM and CAL changes between group sites at any interval time.

TABLE 1 - *Patient characteristics.*

Patients (n)	16
Males (n)	7
Females (n)	9
Mean age (years range)	53 (44-62)
Smokers (n)	0
Teeth/patient (Mean±SD)	23.3±3.5
Test Treatment quadrants (n)	32
Control treatments quadrants (n)	32

n, number of; mean, mean value; SD, standard deviation.

TABLE 2 - *Relative distribution of probing pocket depth categories per patient.*

Pocket depth category	*Test Group n. (%)*	*Control Group n. (%)*
Qualifying sites (PPD ≥5 mm)	470 (43.80%)	443 (40.83%)
Moderate pockets (PPD =5-6 mm)	334 (31.13%)	315 (29.03%)
Deep pockets (PPD ≥7 mm)	136 (12.67%)	128 (11.80%)

Test: HBX/UD-treated sites; control: UD-treated sites; PPD: probing pocket depth at baseline; n: number of sites. The difference between groups was not statistically significant.

Plaque scores (VPI)

At baseline no statistical difference between group sites was presented regarding the Visible Plaque score (VPI). (Test: 26.2%; Control: 28,7%) (Table 3).

Both at T2 and at T3 a statistically significantly lower VPI score was found in the test group sites than in the control group sites [VPII at T2: Test =7,9%; Control 19,4%; (p<0.0001)] [VPII at T3: Test =10,9%; Control 23,7; (p<0.0001)] (*Table 4*).

Bleeding on probing scores (BoP)

In the present study BoP changes represented the primary outcome parameter. At T2 a statistically significantly lower BoP score was found in the test group sites (p<0.0001) and BoP at T2 was reduced by 20.4% in the control group sites and by 42.2% in the test group sites. At T3, a significant BoP value reduction was evident in the control group sites (p<0.0001), and BoP compared with T2 was reduced by 17.3%

TABLE 3 - *Qualifying sites: comparison of clinical parameters at baseline between treatment group sites.*

Clinical Parameters		*Test Group*	*Control Group*	*P value*
VPlI score	n (%)	123 (26.2)	127 (28.7)	NS
BOP score	n (%)	277 (58.9)	294 (66.4)	0.02
GI value	Median (IR) Mean±1 SD	2 (1÷2) 1.59±1.1	2 (1÷3) 1.72±1.1	NS
Prevalence of GI= 0	n (%)	103 (21.9)	90 (20.3)	NS
Prevalence of GI= 1	n (%)	90 (19.1)	59 (13.3)	
Prevalence of GI= 2	n (%)	170 (36.2)	180 (40.6)	
Prevalence of GI= 3	n (%)	107 (22.8)	114 (25.7)	
PPD (mm)	Median (IR) Mean±1 SD	5 (5÷6) 5.54±1.5	5 (5÷6) 5.55±1.4	NS
GM (mm)	Median (IR) Mean±1 SD	0 (0÷1) 0.72±1.4	0 (0÷0) 0.60±1.2	NS
CAL (mm)	Median (IR) Mean±1 SD	6 (5÷7) 6.27±1.9	6 (5÷7) 6.15±1.9	NS

Test group = HBX-UD-treated sites; Control group = UD-treated sites; IR= interquartile range; Mean = mean value; SD = standard deviation.

in the control group sites and by 0.1% in the test group sites. Nevertheless, at T3, a greater BoP score reduction between baseline and T3 was found in the test group sites (42.3%) than in the control group sites (37.7%) with a significant difference in BoP score between the two groups (p<0.0001) (Table 4). In order to rule out a possible confounding action of the oral hy-

TABLE 4 - *Qualifying sites. Clinical parameters at the various examination intervals. Comparison with baseline and between treatment groups sites.*

Parameters		*T1*	*T2*	*P compared to T1*	*T3*	*P compared to T1*
VPlI score						
Control group	n (%)	127 (28.7)	86 (19.4)	NS	105 (23.7)	NS
Test group	n (%)	123 (26.2)	37 (7.9)	P<0.0001	51 (10.9)	P<0.0001
P between groups		NS	P<0.0001		P<0.0001	
BOP score						
Control group	n (%)	294 (66.4)	204 (46.0)	P<0.0001	127 (28.7)	P<0.0001
Test group	n (%)	277 (58.9)	80 (16.7)	P<0.0001	78 (16.6)	P<0.0001
P between groups		0.02	P<0.0001		P<0.0001	
GI value						
Control group	Median (IQR)	2 (1÷3)	1 (0÷2)		0 (0÷2)	
	Mean±1 SD	1.72±1.1	1.2±1.2	P<0.0001	0.81±1.0	P<0.0001
Test group	Median (IQR)	2 (1÷2)	0 (0÷1)		0 (0÷1)	
	Mean±1 SD	1.59±1.1	0.46±0.85	P<0.0001	0.51±0.83	P<0.0001
		NS	P<0.0001		P<0.0001	
Prevalence of GI = 0						
Control group	n (%)	90 (20.3)	194 (43.8)	P<0.0001	245 (55.3)	P<0.0001
Test group	n (%)	103 (21.9)	350 (74.5)	P<0.0001	321 (68.3)	P<0.0001
Prevalence of GI = 1						
Control group	n (%)	59 (13.3)	45 (10.2)	NS	71 (16.0)	NS
Test group	n (%)	90 (19.1)	40 (9.0)	NS	71 (15.1)	NS
Prevalence of GI = 2						
Control group	n (%)	180 (40.6)	125 (28.2)	P<0.0001	92 (20.8)	P<0.0001
Test group	n (%)	170 (36.2)	65 (13.8)	P<0.0001	66 (14.0)	P<0.0001
Prevalence of GI = 3						
Control group	n (%)	114 (25.7)	79 (17.8)	0.004	35 (7.9)	P<0.0001
Test group	n (%)	107 (22.8)	15 (3.2)	P<0.0001	12 (2.6)	P<0.0001
P between groups		NS	P<0.0001		P<0.0001	
PPD (mm)						
Control group	Median (IR)	5 (5÷6)	5 (4÷6)		5 (4÷6)	
	Mean±1 SD	5.55±1.4	5.05±1.6	P<0.0001	4.95±1.7	P<0.0001
Test group	Median (IR)	5 (5÷6)	5 (4÷5)		5 (3÷6)	
	Mean±1 SD	5.54±1.5	4.67±1.4	P<0.0001	4.69±1.7	P<0.0001
P between groups		NS	P<0.0001		0.04	
GM (mm)						
Control group	Median (IR)	0 (0÷0)	0 (0÷0)		0 (0÷0)	
	Mean±1 SD	0.60±1.2	0.60±1.2	NS	0.59±1.2	NS
Test group	Median (IR)	0 (0÷1)	0 (0÷2)		0 (0÷0)	
	Mean±1 SD	0.72±1.4	0.79±1.4	NS	0.64±1.2	NS
P between groups		NS	NS		NS	
CAL (mm)						
Control group	Median (IR)	6 (5÷7)	5 (4÷7)		5 (4÷7)	
	Mean±1 SD	6.15±1.9	5.65±1.7	P<0.0001	5.55±1.9	P<0.0001
Test group	Median (IR)	6 (5÷7)	5 (4÷6)		5 (4÷7)	
	Mean±1 SD	6.27±1.9	5.47±1.9	P<0.0001	5.34±1.9	P<0.0001
P between groups		NS	NS		NS	

T1: First treatment session at baseline, before treatments; T2: Second treatment session 6 weeks from baseline, (i.e. 6 weeks from the first treatment); T3: Third treatment session 3 months from baseline, (i.e. 6 weeks from the repeated treatment); Test group: HBX-UD-treated sites; Control group: UD-treated sites; IQ: interquartile range; Mean: mean value; SD: standard deviation.

giene factor on the final BoP score, the effect of VPI on BOP was assessed both at T2 and at T3. At T2 the BOP was significantly related to treatment (UD vs HBX-UD, p<0.0001) and to baseline BOP (p<0.0001), but not to VPI. The same results were obtained for BOP at T3.

Gingival index value (GI)

At baseline there was no statistical difference between group sites regarding the Gingival Index median values (Test: 2, IQR 1-2; Control: 2, IQR 1-3) (Table 3). Both at T2 (Test: 0, IQR 0-1; Control: 1, IQR 0-2) and at T3 (Test: 0, IQR 0-1; Control: 0, IQR 0-2) the median value was significantly lower in the test group than in the control group (p<0.0001 for both) (Table 4). At baseline, a similar percentage of qualifying site in the test group sites and in the control group sites presented severe inflammation (GI=3) (Table 3). At 6 weeks after treatment, the prevalence of the severely inflammed sites (=3) decreased significantly from 22.8% to 3.2% in the test group sites (p<0.0001) and from 25.7% to 17.8% in the control group sites (p=0.004). At 3 months, 6 weeks after the repeated treatment, 2.6% of sites in the test group and 7.9% in the control group presented severe inflammation, with a significant difference between groups (p<0.0001) (Table 4).

Probing pocket depth (PPD)

At baseline there was no statistical difference in mean PPD between groups sites. (Test: 5.54±1.5 mm; Control: 15.55±1.4 mm) (Table 3). The PPD was significantly lower in the test group sites Both at T2 (Test: 4.67±1.4 mm; Control: 5.05±1.6 mm, p<0.0001) and at T3 (Test: 4.69±1.7 mm; Control: 4.95±1.7 mm, p=0.04) (Table 3). Compared with baseline, PPD showed a significantly greater reduction in the test group sites than in the control group sites, both at T2 [control 0 (0÷1) - 0.51±1.2 mm vs test 1 (0÷2) - 0.87±1.3 mm - P<0.0001] and at T3 [control 0 (0÷2) - 0.60±1.4 mm vs test 1 (0÷2) - 0.85±1.5 mm - P<0.0038] (Table 4).

Gingival margin level (GM) and clinical attachment level (CAL)

Only minimal GM and CAL changes were found at T2 and at T3 in both treatment group sites compared with baseline, and no significant differences were found between treatment group sites (Table 4).

Patient's treatment perception and subjective postoperative hypersensitivity

Based on the described criteria for patient's treatment perception evaluation, 4 patients reported no pain during treatment in the test group sites, 9 patients complained of mild pain and 3 patients of moderate pain. In the control group sites, 3 patients referred no pain, 8 patients complained of light pain, and 5 patients of moderate pain. During the first postoperative week, 8 patients in the control side and 3 patients in the test side complained of postoperative subjective hypersensitivity.

Compared with the control group sides, a significantly lower level of pain was experienced during treatment in the test group sides (p<0.0001), but not for subjective dentinal hypersensitivity during the first postoperative week, even if a trend to a statistical significance was shown (p=0.06, NS) (Table 5).

Time spent for treatment

On the basis of the described criteria for treatment completion, during the first treatment session, the mean time used for ultrasonic debridement was 22±2.9 minutes in the control group sites and 18±.3.8 in the test group sites (p=0.0011). During the second treatment session, the respective values for subgingival instrumentation were 20.6±2.8 minutes in the control group sites and 16.1±3.2 in the test group sites (p=0.002). In the test group sites the time needed for the subgingival administration of the test substance was 5,1±0,8 minutes

TABLE 5 - *Patients' perception of treatment.*

Treatment effects	*Test*	*Control*	*p*
Level of pain experienced during treatment¥ (median e IQR)*	30 (27.5-35)	40 (30-50)	p<0.0001
Subjective dentinal hypersensitivity experienced during the first postoperative week [n (%)]	3 (18.8%)	8 (50%)	NS

VAS scale 1 - 100; Test: HBX-UD-treated sites; control: UD-treated sites; mean: mean value; SD: standard deviation; median: median value; IQR: interquartile range - n: number of patients.

TABLE 6 - *Treatment time (minutes) assessed for the two treatment approaches during the first treatment session and at the 6 weeks repeated-treatment session.*

	Test	*Control*	*p*
First session			
Hybenx placement	5.1±0.8	=	0.0011
Debridement	17.9±3.8#	22.2±2.9	NS
1st session Total time	22.6±3.6	22.2±2.9	
Second session			
Hybenx placement	4.8±0.6	=	
Debridement	16.1±3.2	20.6±2.8	0.0002
2nd session Total time	20.1±3.2	20.6±2.8	NS
Total time	44.1±7.7	42.8±5.5	NS

Test: HBX/UD-treated sites; control: UD-treated sites.

during the first treatment session and 4.8±0.6 during the second session. Taking together the time served for treatment at baseline and at 6 weeks, the treatments in the control group sides required a total of 42.8±5.5 minutes, while the total treatment time in the test group sides was 44.8±.7,7. The time difference between the two treatment approaches did not result statistically significant (Table 6).

Microbiological results

The microbiological results are reported in Table 7. To allow evaluation of total cultivable counts changes in the aerobic (AER) and anaerobic (ANAER) population, at various interval time examinations, microbiological samples were collected from 38 preselected qualifying sites respectively in test and control treatment group sites. Total bacterial load differences between groups were not significant at baseline. At the end of the first treatment session (T1c), both procedures caused a statistically significant reduction of aerobic and anaerobic bacterial load compared with baseline (p<0.0001 for both).
After 6 weeks (T2) significantly reduced anaerobic bacterial loads were present in both treatment group sites compared with baseline (p<0.0001 for test group, p=0.01 for control group), while for aerobic bacterial loads a mild significant reduction was found for test group (p=0.02), but no reduction in the control group. Repeated treatment administration did not provide any changes between T2 and T3 in the test group sites.
At T3 compared with baseline, both treatments group sites presented similar significant reduction in anaerobic bacterial load compared with baseline (p=0.0001 for control group, p<0.0001 for test group), while no differences were presented in aerobic bacterial load.

TABLE 7 - *Qualifying sites. Microbiological results at the various examination intervals. Comparison with baseline and between groups sites (mean value and standard deviation).*

	T1a	*T1b*	*P compared to T1a*	*T1c*	*P compared to T1a*	*T2*	*P compared to T1a*	*T3*	*P compared to T1a*
Aerobic Log_{10} (Ufc/ml)									
Control group	6.14±1.15	=	=	3.51±0.83	P<0.0001	5.85±1.08	NS	6.57±0.80	NS
Test group	6.28±0.75	3.27±0.88	P=0.0003	1.28±0.91	P<0.0001	5.38±1.20	0.02	5.97±0.83	NS
P between groups	NS	=	=	P<0.0001	=	NS	=	NS	=
Anaerobic Log_{10} (Ufc/ml)									
Control group	6.13±1.17	=	=	3.17±1.06	P<0.0001	4.84±1.43	0.01	4.24±1.28	0.0001
Test group	5.84±0.93	2.57±0.77	P=0.0003	1.08±1.00	P<0.0001	3.48±1.40	P<0.0001	3.25±1.33	P<0.0001
P between groups	NS	=	=	P<0.0001	=	0.018	=	NS	=

T1a: First treatment session at baseline before treatments; T1b: First treatment session, immediately after HBX administration and before UD; T1c: First treatment session, immediately after UD administration; T2: Second treatment session, 6 weeks from baseline, (i.e. 6 weeks from the first treatment); T3: Third treatment session, 3 months from baseline, (i.e. 6 weeks from the repeated treatment); Test group: HBX-UD-treated sites; Control group: UD-treated sites; Mean: mean value; SD: standard deviation.

When results were compared between groups, intergroup statistical analysis demonstrated that at T1b in the test group sites the mere administration of HBX provided similar bacterial loads reductions than UD at T1c in the control group sites (Figure 2), and when results were compared between groups at T1c, the HBX-UD treatment provided a significantly two times greater reduction in both aerobic and anaerobic species than the UD.
At T2 significant lower levels were found for the anaerobic bacterial load in test group sites (p=0.018), while no differences between group sites were detected for aerobic bacterial load.

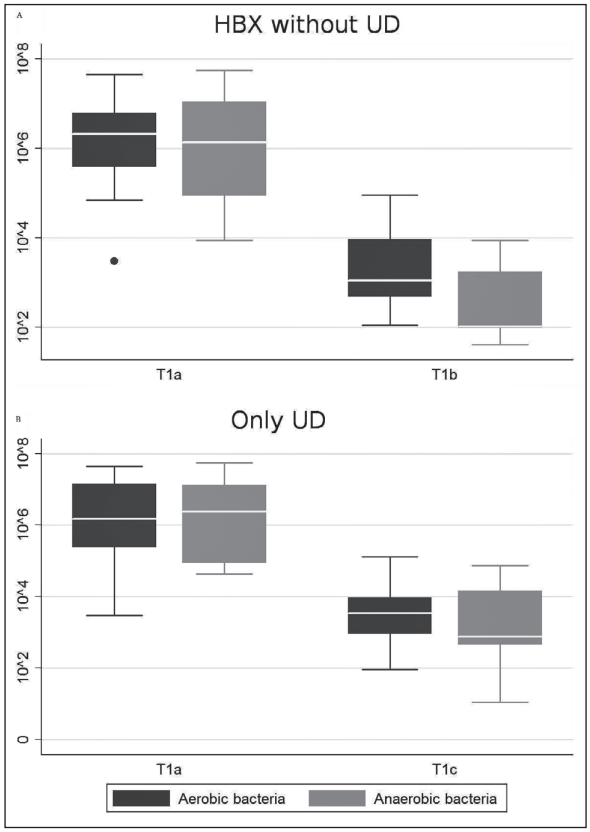

FIGURE 2 - *Comparison between aerobic and anaerobic total bacterial load reductions (log10) assessed after HBX subgingival administration alone in the test group sites (A) and after UD administration in the control group sites (B). T1a: First treatment session, at baseline, before treatments. T1b: First treatment session, immediately after HBX administration and before UD, in the test group sites. T1c: First treatment session, immediately after UD administration in the control group sites.*

After 3 months and two repeated treatment administrations, both treatment groups presented statistically significant reductions in anaerobic bacterial load compared with baseline, but analysis failed to demonstrate statistical differences between groups.

DISCUSSION

The main goal of the initial approach to treatment of chronic periodontitis is to provide and maintain adequate infection control in supra and subgingival areas.
The aim of this 3-month randomized prospective controlled split-mouth study was to evaluate if the topical administration of a desiccant solution (HBX) may improve the clinical and microbiological outcome of the standard ultrasonic instrumentation in patients with chronic periodontitis.
To obtain very reproducible time and operative conditions with respect to a hospital setting where it has to be provided for the greatest number of patients with the best possible supra and subgingival ultrasonic debridement, only 50 minutes for each treatment session were allowed for each full mouth treatment, and only standard tips were utilized as inserts for both supra and subgingival ultrasonic instrumentation to treat all the sites.
Since there is no evidence in the literature that a subgingival irrigation with saline solution or with water before UD may enhance the clinical or microbiological outcome results, and because the UD procedure provides continuous irrigation, no placebo was used on the control side. To evaluate a possible additive effect, repeated treatments were provided after 6 weeks. Clinical and microbiological parameters were evaluated at baseline, after 6 weeks and after 3 months. (Clinical and microbiological results are reported respectively in in Tables 3 and 4).
The short-term results demonstrated that after 6 weeks both treatment procedures provided significant BoP reduction after 6 weeks compared with baseline (P<.001), and that significantly greater BoP score reductions was provided in the test group sites from the UD+HBX treatment than in the control group sites from the standard UD: after a single treatment ses-

sion BoP resulted two times more reduced in the test group sites than in the control group sites.
When the results were evaluated after three months UD+HBX treatment still presented better overall performances in terms of gingival inflammation reduction.
It is noteworthy that even if repeated treatment provided significant effects only in the control group sites and no changes in the test group sites, superior BoP reductions were still found at T3 in the test group sites (Table 4).
As reported in the literature (Paolantonio *et al.,* 2009), BOP score offers the advantage of comparability with other studies, but presents disadvantages to determine differences in inflammation severity. Sites that bled profusely at baseline show an unvaried BOP score even in case of remarkable reduction in inflammation and poor bleeding after treatment.
Hence, GI was evaluated to allow a better understanding of the changes in the severity of gingival inflammation between group sites. Results showed that after six weeks not only the prevalence of bleeding sites but also the severity of inflammation signs was significantly more reduced in the test group sites than in the control group sites (Table 4), and a statistically greater percentage of qualifying sites improved their Gingival Index from a GI=3 to a GI=0 value (test: 56.1%; control: 25.4%, $p<0.0001$). When the results were evaluated after three months, UD+HBX treatment presented better overall performance in terms of gingival inflammation reduction.
Even if in the present study all patients presented good oral hygiene standard and good compliance throughout the experimental period, significantly lower VPI scores were found in the test sides than in the control sides at any interval examination. The possible confounding action of the oral hygiene factor on the final BoP score was evaluated with a logistic multivariate models with BoP at T2 and T3 as dependent variables, VPI was added as a regressor and no effect on BoP was evident at T2, and at T3.
HBX is a strong contact desiccant, so it might be hypothesized that HBX application on exposed dentin could result in increased pain and sensitivity. When patients were asked to refer their perception of the treatment, according to the literature (Leonhardt *et al.,* 2007, Wennstrom *et al.,* 2005) they indicated UD to be a well-tolerated procedure, but the majority of them experienced lower levels of pain and less incidence of subjective dentinal hypersensitivity in the test sides after UD+HBX combined treatment compared to those treated on the control side with UD alone (Table 5).
In addition, time spent for the full mouth administration of HBX added approximately 5 minutes per treatment session, and did not markedly affect the total treatment time in the test side versus the control side (Table 6).
On the other hand, when PPD and CAL reductions were evaluated as indicators of successful clinical outcomes, in contrast with the positive effects shown on the gingival inflammation signs, very poor results were provided by both therapy methods in both treatment group sites at any interval times (Table 4). Probably the use of thin periodontal probe-like inserts might improve the mechanical efficacy of ultrasonic subgingival debridement (Clifford *et al.,* 1999, Dragoo, 1992) especially in deep sites, and in the same way, better results might have been provided with more time available for the instrumentation, but this was not allowed by the experimental conditions of this study.
However, the possibility of obtaining greater gingival inflammation reduction after only a single treatment and at the same time maintaining the results over time may have a clinical meaning, especially for patients that need the best results in the shortest and painless as possible treatment session.
Together with other variables, BoP is an essential part of a number of periodontal risk assessment methods (Lang and Tonetti, 2003, Page *et al.,* 2002, Trombelli *et al.,* 2009) and lack of bleeding on probing is a predictive factor for future attachment stability and tooth survival (Lang *et al.,* 1990, Matuliene *et al.,* 2010), even if taken as a single clinical indicator (Joss *et al.,* 1994).
If a full-mouth BoP prevalence of ≤25% is considered in the literature to be the cut-off point below which it is reasonable to expect a significantly lower risk of disease progression, it is worthy of attention that even under the limitations of this study, UD+HBX treatment reached this desirable treatment endpoint after only a

single treatment session and maintained stable results up to 3 months, while UD did not reach a similar result even after 3 months and two repeated instrumentations.
To investigate the antibacterial effects and the consequent host responses 37 and 36 pockets have been identified for subgingival plaque examination with cultural methods in test and control group sites respectively. Differences in both anaerobic and aerobic total bacterial counts were not significant at baseline between group sites.
As expected from the literature (Leonhardt *et al.,* 2007, Miyazaki *et al.,* 2003, Paolantonio *et al.,* 2009), the present study results demonstrated that the ultrasonic instrumentation provides a significant short-term bactericidal efficacy and that a gradual bacterial regrowth takes place after 1.5 months.
When the adjunctive effect of an antiseptic solution of 0.1% Povidone-iodine was evaluated in the literature, no additional benefit was found in the detection frequencies of the main periodontal pathogens after 1 month and 3 months compared with baseline (Del Peloso Ribeiro *et al.,* 2006). In the present study, HBX was demonstrated to possess a bactericidal effect even if topically administered as monotherapy, providing in the test group sites the same decrease in bacterial loads found in the control group sites after UD administration.
When it was adjunctively administered to the UD, the immediate and short-term bactericidal efficacy of the ultrasonic instrumentation was considerably improved. After 6 weeks, even if in accordance with the literature, a substantial bacterial regrowth occurred in both treatment groups, a significantly greater reduction of the anaerobic bacterial load was still presented in UD+HBX treated sites.
In accordance with the clinical results, the repeated treatment rendered at T2 proved effective especially in the control group sites, where it provided an additional antibacterial effect which allows a better bacterial control at T3.
Consequently at the 3 month examination in both treatment groups significant reductions in anaerobic bacterial loads were found compared with baseline, and it may be suggested that under the experimental conditions of this study, repeated treatment sessions may not only allow better clinical results but might also provide better bacterial control.

CONCLUSIONS

This 3-month clinical and microbiological study supports the conclusion that the supra- and sub-gingival topical administration of a desiccant agent with anti-biofilm properties enhances the bactericidal efficacy of the subgingival ultrasonic instrumentation.
Under the limitations of this study, the test treatment resulted in a well, if not better, tolerated procedure for the patients, and, in comparison with the control treatment, it provided a greater reduction in gingival inflammation and anaerobic bacterial load, while no significant effects on PPD, CAL and GM were detected.
On the basis of these outcomes, further studies with different design (i.e.: topical administration of the agent not only before debridement but also after debridement to assure optimal cleaning; the use of slim periodontal tips in the deep sites; no limitations in time allowed for treatment) and larger cohorts and samples are auspicated would be expected to further support the conclusions of this study.

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