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Effectiveness of ultrasonic instruments in the therapy of severe periodontitis: a comparative clinical-microbiological assessment with curettes

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SUMMARY

Patients with deep periodontal pockets were treated with either Vector® System (TG) or manual instruments (CG). Clinical assessments by supragingival plaque (PL+), gingival index (GI), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL) and subgingival plaque collection for microbiological analysis were made prior to and after treatment. Multiplex Polymerase Chain Reaction was used to determine the presence of *Actinobacillus actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Prevotella intermedia, Porphyromonas gingivalis, Tannerella forsythensis* and *Treponema denticola*.
GI, PD, CAL and the number of BOP+ sites underwent a significant reduction over time in both groups. When compared to baseline, the pair-wise analyses showed significantly lower PD and CAL at 6 months in the CG and significant reductions in the GI, PD, CAL and a number of BOP+ sites at 3 and 6 months in the TG.
For microbiological results, significant reductions were seen for *C. rectus* and *P. gingivalis* in the CG and for *T. forsythensis*, *E. corrodens* and *T. denticola* in the TG. The total bacterial count underwent a reduction in both groups.
Both ultrasonic and manual debridement are equally effective in non-surgical periodontal therapy of severe periodontitis in terms of clinical and microbiological effects.

KEY WORDS: ultrasonic subgingival debridement, non-surgical periodontal therapy, periodontophatic bacteria, multiplex PCR analyses.

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INTRODUCTION

Scaling and root planing are the most commonly utilized periodontal therapies to obtain clinical healing of periodontal involved sites (Obeid *et al.*, 2004) and since the vast majority of clinical trials relating to periodontitis therapy have been conducted using curettes and/or scalers, manual instrumentation is generally regarded as the

gold standard (Cugini *et al.,* 2000; Tunkel *et al.,* 2002). Despite this, manual scaling and root planing can often be difficult and time-consuming due to the complex and unfavourable root morphology when working blindly at deep pocket sites (Obeid *et al.*, 2004; Serino *et al.*, 2001).
Taking these factors into consideration, ultrasonic scalers have come to be widely used in recent years because of their simplicity of use, the time advantage, a reduction in physical effort and physical stress of the operator (Obeid *et al.*, 2004; Kishida *et al.*, 2004).
Several studies have reported on the clinical and microbiological effects obtained by old generation ultrasonic devices and the results obtained are similar to those achieved by manual instruments (Baehni *et al.*, 1992; Cobb, 2002; Sculean *et al.*, 2004). Furthermore, several studies have reported an increased efficiency of ultrasonic/sonic subgingival debridement since they require less time than manual instruments to obtain similar results (Tunkel *et al.,* 2002).
An innovative ultrasonic instrument, the Vector® System (Dürr Dental GmbH & Co. Bietigheim-Bissingen, Germany), is now available for scaling and root planing in periodontal therapy. This device uses a different type of energy transmission than conventional ultrasonic systems and its function is based on a hydrodynamic flow technique combined with fine polishing particles. Preliminary clinical results have shown that the use of this innovative ultrasonic instrument can reduce pain during treatment of periodontal lesions. This leads to clinical improvements comparable to those obtained with conventional manual instruments, and is capable of producing smooth root surfaces with the preservation of more cementum (Sculean *et al.*, 2004; Braun *et al.*, 2003).
On the contrary, the effect on putative periodontal pathogens after treatment with Vector® has not yet been studied. Bacteriological tests in periodontology have been available to clinicians since the end of the 1980s. Several studies report that to integrate a well-developed and sensitive microbiologic detection system in periodontal diagnostics would be of help in the diagnosis and treatment of periodontal diseases and can also help in verifying the efficiency of the treatment (Armitage, 2004; Umeda *et al.*, 2004).
The objectives of nonsurgical therapy are the reduction of the bacterial load, the alteration of the microbial composition towards a flora associated with health. Monitoring patients by microbiologic tests during treatment can be an aid in understanding whether this is efficient and whether patients respond to therapy. As a microbiologic detection system, PCR-based approaches provide a sensitive and reliable method for identification and monitoring treatment of periodontal pathogens.
To further underline the relationship between ultrasonic therapy and periodontal tissue healing, the present study evaluated the clinical and microbiological effectiveness of scaling and root planing by a power-driven mechanism (Vector® System) compared to manual instruments in the treatment of deep pockets (probing depth $\geq$6 mm) with a split-mouth design.

MATERIAL AND METHODS

Study population and design

Eighteen non-smoking patients, 7 males and 11 females (average age 40.8 ±3.9 years) were chosen in this study. Each patient gave informed consent to participate in the study by signing a consent form, and the study protocol was approved by the Ethical Committee of the University Medical Faculty.
The subjects had to comply with the following criteria:

1) positive for diagnosis of mild-to-severe chronic periodontitis;
2) good general health according to their medical history;
3) negative for the use of any antibiotic or anti-inflammatory drugs within the three months preceding the beginning of the study;
4) negative for periodontal therapy within 1 year preceding the beginning of the study
5) experimental sites (test and control) localized in the interproximal position of two different teeth in the same subject (split-mouth design);
6) probing depth (PD) values equal to or more than 6 mm in the experimental sites;
7) difference of PD in the experimental sites (test and control) not exceeding 2 mm;
8) presence of at least ten teeth for each dental arch. Pregnant or nursing females were excluded from the study.

Oral hygiene instructions (OHI), consisting of Bass' brushing technique, the correct use of dental floss and an interdental brush, were given to all participants before the beginning of the study. The same OHI were again pointed out throughout the study. Finally, subjects were not allowed to take any antibiotics and anti-inflammatory drugs, or chlorhexidine-based mouth rinses throughout the study.

Seven days before baseline, complete supragingival plaque and calculus removal was assessed. At baseline, the sites were assigned into two experimental groups. The first, Test Group (TG), was treated by means of a new ultrasonic device, whereas the second, Control Group (CG), received scaling and root planing by means of manual instruments. Only at baseline, after clinical measurements and after the collection of subgingival plaque samples, did each site receive subgingival instrumentation under local anaesthesia for ten minutes.

Clinical and microbiological monitoring were performed at baseline and at 3 and 6 months after the end of treatment (Fig. 1). One month after baseline, clinical examinations of supragingival plaque (PL+), gingival index (GI) and of subgingival plaque samples were carried out.

Clinical procedures and subgingival plaque collection

Full-Mouth Plaque Score (FMPS) and Full-Mouth Bleeding Score FMBS were recorded as the % of tooth surfaces (mesio-, distal-, buccal, palatal/lingual surfaces in each tooth) having a presence of supragingival plaque or bleeding within 15 seconds after probing with a 20 g controlled-force probe (Vivacare TPS Probe, Vivadent, Schaän, Lichtenstein). Moreover, in each experimental site, the clinical examinations consisted of recording the presence of:

1) supragingival plaque (PL+), assessed by visual criteria;
2) gingival index (GI);
3) probing depth (PD);
4) clinical attachment level (CAL);
5) gingival bleeding within 15 s after probing (BOP+) with a 20 g controlled-force probe;
6) gingival crevicular fluid sample (GCF) for microbiological analysis.

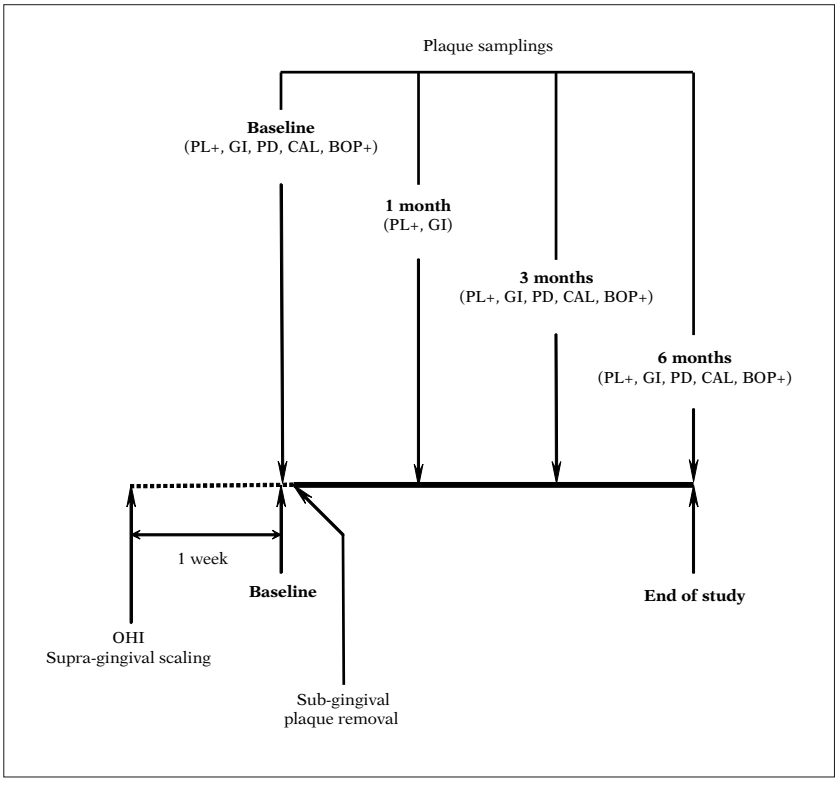

FIGURE 1 - *Diagram of the study design. See text for details.*

PD and CAL measurements were assessed after subgingival plaque sampling to avoid possible alteration of microbiological analysis.
A resin stent was built for each site to standardize probing depth and CAL measurement. Using the stent as a guide, the periodontal probe was inserted into the pocket and PD (using the gingival margin as reference), and CAL (using the most apical end of the stent as reference) were recorded, as previously described by Camargo *et al*., (2000).
Subgingival plaque was collected for microbiological evaluation as follows: the sites were first isolated with cotton rolls and after removal of supragingival plaque when present with a sterile curette. The gingival surface was dried with a gentle, sterile oxygen-free-CO_2 gas flow; the plaque samples were then obtained by insertion of four standardized #30 sterile paper points (Krugg, Buccinasco, Milan, Italy) into the deepest part of each periodontal pocket, left in situ for 15 s for saturation. Two paper points were transferred into sterile eppendorf for the PCR analyses; and two paper points were transferred into sterile eppendorf containing 1 ml of freshly prepared Reduced Transport Fluid (RTF).

MICROBIOLOGICAL PROCEDURES

Culture conditions

Patient samples were dispersed by vortexing for 60 s, and each sample was subjected to a series of 10-fold dilutions (to 10^{-4}) in 0.1 M phosphate buffer. Aliquots of 100 µl from each dilution were spread onto Columbia Blood Agar (CBA) plates (Oxoid Italia SpA, Garbagnate Milanese, Milan, Italy) for total anaerobic viable count, recorded as the count of colony forming units *per* ml (CFUs/ml) on the growth plate. The plates were incubated at 37°C for 7 days in an anaerobic chamber (80/10/10, $N_2/H_2/CO_2$; Don Whitley Scientific Ltd; International PBI SpA, West Yorkshire, UK).

DNA-extraction

Nucleic acids were extracted within 24 to 48 hours from specimens collected. The samples were vortex-mixed and centrifuged to collect the cells. The pellet was suspended in 300 µl of lysis buffer (50 mM Tris, 10 mM EDTA and 10% SDS) plus lysozyme (5 mg/ml) and incubated at 37°C for 1 hour. Then proteinase-K was added and after 1-hour incubation at 65°C the DNA was extracted with phenol and chloroform-isoamyl alcohol treatment. Nucleic acids were precipitated in alcohol, washed with 70% (vol/vol) alcohol and resuspended in sterile water. The DNA extracted from each sample was assayed by multiplex PCR, for the detection of *Actinobacillus actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Prevotella intermedia, Porphyromonas gingivalis, Tannerella forsythensis* and *Treponema denticola*.

PCR-detection

The multiplex PCR was performed by using specific primers for the 16S rRNA gene of each bacterium. Table 1 lists the PCR primers used in the current study. PCR amplification reactions were carried out in a reaction mixture in a final volume of 100 µl consisting of 10 µl of DNA sample, and 90 µl of reaction mixture containing 30 pmol of each primer, 200 µM of a mixture of deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 1 x PCR buffer (10 mM Tris-HCl, pH 8.0), 50 mM KCl, 2.5 U Hot Start Taq™ DNA Polymerase (Quiagen SpA, Milan, Italy). The PCR protocol was as follows: 98°C for 15 min followed by 40 cycles of 95°C for 30 s, 60°C for 1 min, 72°C for 1 min, and a final step of 72°C for 10 min.
PCR amplification was performed in an iCycler System (Bio-Rad Laboratories Srl, Segrate, Milan, Italy). Amplicons were detected by electrophoresis of 20 µl of samples from each PCR tube in a 2 % agarose gel in TAE (Tris-Acetate-EDTA buffer) for 2 h at 80 V. The amplification products were visualized and photographed under a UV light transilluminator (Gel Doc 2000-Bio-Rad) after 30 min of ethidium bromide (1 µg/ml) staining. The molecular sizes of the amplicons were determined by comparison to a commercial DNA molecular weight marker (number VIII**,** Roche Diagnostics SpA, Milan, Italy).
The frequency of sites positive for each microbiota was reported.

DATA ANALYSIS

The Statistical Package for Social Sciences software (SPSS version 8.0, SPSS® Inc, Chicago,

TABLE1 - *Sequences and expected product size for PCR primers (Slots et al.,1995; Ashimoto et al., 1996; Santangelo et al., 2004).*

Primer pairs 5'-3'	*Amplification length*
Actinobacillus actinomycetemcomitans GCT AAT ACC GCG TAG AGT CGG ATT TCA CAC CTC ACT TAA AGG T	443 bp
Tannerella forsythensis (Bacteroides forsythus) GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	641 bp
Campylobacter rectus TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT	598 bp
Fusobacterium nucleatum GAA GAA ACA AAT GAC GGT AAC AAC GTC ATC CCC ACC TTC CTC CT	705 bp
Eikenella corrodens CTA ATA CCG CAT ACG TCC TAA G CTA CTA AGC AAT CAA GTT GCC C	688 bp
Porphyromonas gingivalis AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	404 bp
Prevotella intermedia AAC GGC ATT ATG TGC TTG CAC CTC AAG TCC GCC AGT TCG CG	589 bp
Treponema denticola TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	316 bp
Ubiquitous primer GAT TAG ATA CCC TGG TAG TCC AC CCC GGG AAC GTA TTC ACC G	602 bp

Illinois, USA) was used to perform the data analysis. Parametric analyses were performed after the required assumptions were verified. The number of tooth sites PL+ and BOP+ were treated as dichotomous data while the GI as ordinal data, and the PD and CAL as continual data. The significant differences in PL+ and BOP+ between the groups at each time point were assessed by means of the McNemar analysis, while the differences over time within the groups were assessed with the Cohrane test. When significant differences were found a further Bonferroni-corrected McNemar analysis was performed as pairwise comparisons between each time point and the baseline. The differences in GI between the groups at each time point were assessed with the Wilcoxon paired sign rank test, while the differences over time within the groups were assessed by the Friedman test followed by a Bonferroni-corrected Wilcoxon paired sign rank test as pair-wise comparisons between each time point and the baseline. Significant differences, in both the PD and CAL, between the groups at each time point were assessed by the paired t-test, while the differences over time within the groups were assessed by the Repeated measure one-way analysis of variance (ANOVA) followed by a Bonferroni-corrected paired t-test as pairwise comparisons between the 3 and 6 months examinations and the baseline.

Differences in the number of sites positive for each microbial species, between the groups by time point were tested by McNemar test. Furthermore, within the experimental groups differences in data over time were processed by a Cochran test followed by a Bonferroni-corrected McNemar test as pair-wise comparisons.
A probability of $p<0.05$ was accepted for rejection of the null hypothesis.

RESULTS

Clinical outcomes over time in the two experimental groups are summarized in Table 2. FMPS and FMBS from all subjects participating in the study remained ≤20% throughout the study (data not shown). At baseline, all clinical parameters were similar among the groups, without any statistically significant differences. The number of PL+ sites were similar throughout the study with no significant differences in both the groups. On the contrary, GI, PD, CAL and the number of sites BOP+ underwent a significant reduction over time in both groups. The pair-wise analyses showed significant lower PD and CAL at 6 months as compared to those at baseline in the CG. The BOP also underwent a reduction over time in this group although not at a statistically significant level. In the TG, the pair-wise analyses showed significant reductions in the GI, PD, CAL and the number of BOP+ sites at 3 and 6 months as compared to baseline values. A statistically significant decrease of BOP+ at 3 months and PD at 6 months in the test group compared to control group was observed.
Microbiological findings are shown in Table 3 and Figure 2. Within the CG, the number of sites positive for *C. rectus* and *P. gingivalis* underwent a significant reduction while within the TG similar reductions were seen for *T. forsythensis*, *E. corrodens* and *T. denticola*. In particular, the pair-wise analysis showed a significant reduction in the number of sites positive for *T. denticola* at each time point as compared to the baseline value.
The total bacterial count underwent a reduction in both the groups but only in the TG did such changes reach statistical significance. However, no statistically significant differences between the groups were detected at any time point.

DISCUSSION

The term mechanical therapy refers to the debridement of the roots by a time-consuming and difficult procedure in removing plaque, endo-

TABLE 2 - *Number of sites with presence of supragingival plaque (PL+) and bleeding on probing (BOP+), and mean gingival index (GI), probing depth (PD) and clinical attachment level (CAL) (±SD) in the different experimental groups over time.*

Variable	*Baseline*			*1 month*			*3 months*			*6 months*			*Difference over time*	
	CG	*TG*	*Diff.*	*CG*	*TG*	*Diff.*	*CG*	*TG*	*Diff.*	*CG*	*TG*	*Diff.*	*CG*	*TG*
PL+	4	3	NS	3	3	NS	3	5	NS	0	2	NS	NS	NS
GI	1.1 (±0.2)	1.1 (±0.4)	NS	0.8 (±0.5)	0.8 (±0.6)	NS	0.8 (±0.4)	0.6† (±0.5)	NS	0.8 (±0.4)	0.6* (±0.5)	NS	p<0.05	p<0.05
PD (mm)	8.1 (±1.2)	8.1 (±1.6)	NS	-	-	-	8.1 (±1.8)	7.1* (±2.0)	NS	6.4† (±2.2)	5.4† (±2.1)	p<0.05	p<0.001	p<0.001
CAL (mm)	13.2 (±2.4)	13.7 (±1.7)	NS	-	-	-	13.2 (±2.8)	12.7* (±2.6)	NS	11.3† (±2.9)	11.0† (±2.5)	NS	p<0.001	p<0.001
BOP+	18	18	NS	-	-	-	15	10*	p<0.05	13	11*	NS	p<0.05	p<0.01

CG, Control Group; TG, Test Group. Each group contained n=18. Diff., statistically significant difference between the groups within each time point. Significant difference compared to corresponding baseline value: *p<0.05, †p<0.01. NS, not statistically significant difference.

TABLE 3 - *Number of sites positive for the presence of each bacterial species over time in the different experimental groups.*

Bacterial species	*Baseline*			*1 month*			*3 months*			*6 months*			*Difference over time*	
	CG	*TG*	*Diff.*	*CG*	*TG*	*Diff.*	*CG*	*TG*	*Diff.*	*CG*	*TG*	*Diff.*	*CG*	*TG*
Actinobacillus actinomycetemcomitans	7	6	NS	8	10	NS	5	5	NS	11	8	NS	NS	NS
Tannerella forsythensis	15	13	NS	14	16	NS	14	9	NS	16	11	NS	NS	p<0.05
Campylobacter rectus	14	8	NS	12	12	NS	8	8	NS	14	8	NS	p<0.05	NS
Eikenella corrodens	11	14	NS	11	9	NS	12	8	NS	10	6	NS	NS	p<0.05
Fusobacterium nucleatum	12	13	NS	14	14	NS	15	10	NS	11	10	NS	NS	NS
Porphyromonas gingivalis	3	2	NS	5	3	NS	1	0	NS	0	1	NS	p<0.05	NS
Prevotella intermedia	4	4	NS	9	8	NS	8	3	NS	8	5	NS	NS	NS
Treponema denticola	6	13	NS	8	5*	NS	11	6*	NS	8	5*	NS	NS	p<0.01

CG, Control Group; TG, Test Group. Each group contained n=18. Diff., statistically significant difference between the groups within each time point. Significant difference compared to corresponding baseline value: *p<0.05. NS, not statistically significant difference.

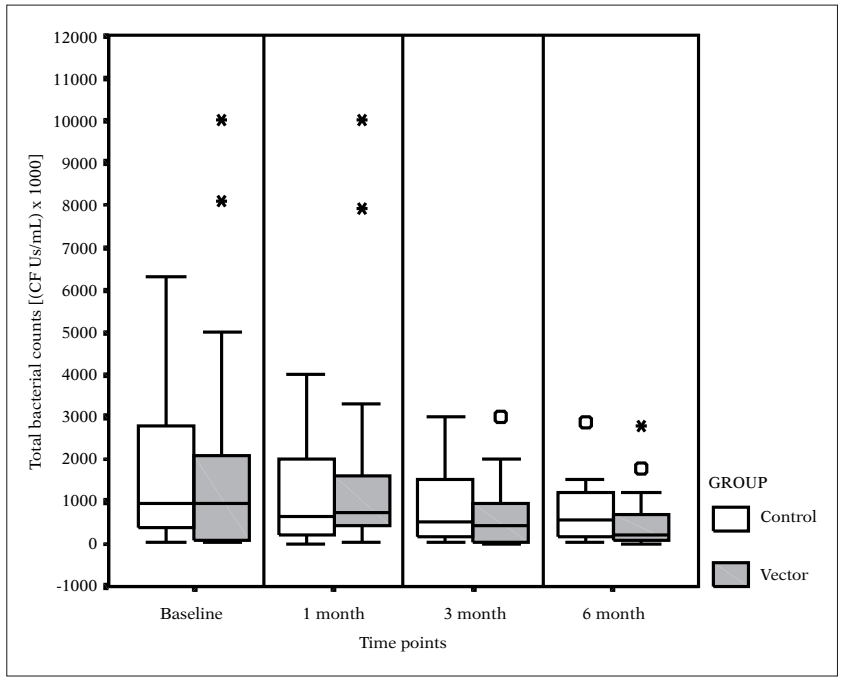

FIGURE 2 - *Boxgraph of total bacterial counts (CFUs/mL) from the sub-gingival plaque in the different experimental groups over time. Each group contained n=18. Statistically significant decrease in the total bacterial counts over time within the TG (Friedman test, p<0.05).*

toxin, calculus and other plaque-retentive local factors. This procedure includes scaling and root planing by manual instruments and/or machine driven instruments, e.g., ultrasonic and sonic scalers. It requires a great deal of stamina on the part of both operator and patient. Success is highly dependent on the skill of the clinician and on attention to improper instrumentation. Numerous studies have reported beneficial results from both treatments in both clinical and microbiological parameters (Obeid *et al.*, 2004; Tunkel *et al.*, 2002). However, several studies have demonstrated that manual instrumentation usually takes from 20 to 50% longer than that of sonic/ultrasonic scalers in achieving the same clinical end-points (Drisko, 2001). As probing depth increases, manual instruments become less effective in removing the cause of the problem (Cugini *et al.*, 2000). This led to the development of power-driven mechanical instruments that have enhanced the ability of the operator to reach into furcations more effectively and to penetrate the depth of the pocket more easily (Clifford *et al.*, 1999).

The use of Vector®-system for cleaning periodontal lesions can reduce pain and discomfort compared not only to conventional methods (Braun *et al.*, 2003) but also to other ultrasonic systems. Unlike other conventional ultrasonic systems, the reduction of 3-D oscillatory effects of the working tips increases the patient's compliance during non-surgical periodontal therapy and recall. Moreover, the absence of aerosol effect minimizes the contamination risk for the operator (Sculean *et al.*, 2004).

The aim of this study is to evaluate the effectiveness of both manual and ultrasonic treatments (Vector®-system) in deep pockets (probing depth ≥6 mm). Both treatments have showed statistically significant PD reduction and CAL gain. The results regarding PD reduction and CAL gain obtained after the use of manual instruments are in agreement with several previous investigations (Serino *et al.*, 2001; Sculean *et al.*, 2004; Drisko, 2001). So far, there are only two studies which report on the clinical effect following the Vector®-system treatment (Sculean *et al.*, 2004; Sculean *et al.*, 2003). They showed a mean CAL gain of 1.5 ± 0.5 and 0.6 ± 0.4 mm at initially deep sites 6 months after treatment, respectively. Our CAL gain value of 2.7 ± 0.8 was higher than that from the previous studies. Although a statistically significant decrease of BOP+ at 3 months and PD at 6 months in the test group compared to the control group was observed, we were unable to provide definite data about higher clinical effectiveness of the Vector®-system compared to manual instruments.

Previous investigations (Sculean *et al.*, 2004; Sculean *et al.*, 2003) are limited because they did not use any microbiological monitoring. The inclusion of an additional microbiological test significantly enhanced the diagnostic characteristics of clinical examinations. Our study also used microbiological monitoring to evaluate the efficacy of periodontal therapy which is directly related to the ability of the treatment to lower levels and/or the prevalence of one or more pathogenic bacterial species. The average clinical changes in the present study were accompanied by specific changes in the average levels of the subgingival microbiota (Table 3).

Both treatment modalities resulted in a reduction of the total bacterial count, although in the CG it was not statistically significant, probably due more to a lack of statistical power than a real lack in the efficacy of the treatment (Figure 2). The treatment results in a reduction of some of the putative periodontal pathogens tested, but the majority of the bacteria were still detected during post therapy. This is in agreement with other reports which showed that manual instrumentation is able to lower the number of selected periodontal pathogens, as *P. gingivalis, T. forsythensis* and *T. denticola* but is unlikely to eliminate these species from any subject (Cugini *et al.*, 2000; Cobb, 2002; Apatzidou *et al.*, 2004; Haffajee *et al.*, 1997). Manual instrumentation decreases the population of Gram-negative bacteria and allows for an increase in the population of Gram-positive microbes. This shift is usually associated with an improvement in clinical parameters, such as decreased PD or bleeding on probing (BOP) (Haffajee *et al.*, 1997; Slots *et al.*, 1979).

Few studies have reported the effect of ultrasonic instruments on the subgingival bacteria. They are in agreement when demonstrating that the manual, sonic and ultrasonic treatments cannot effect the complete removal of subgingival bacteria and achieve similar clinical and microbiological results (Cobb, 2002; Umeda *et al.*, 2004).

In our study, the ultrasonic treatment lowered

the proportion of *T. forsythensis*, *T. denticola* and *E. corrodens* together with an improvement in clinical parameters, such as a reduction in GI, PD, CAL and a number of BOP+ sites. These bacteria have been noticed in deep pockets by a number of studies(Haffajee *et al.*, 1997; Socransky *et al.*, 1998), whereas their prevalence is lower at shallow and periodontally healthy sites (Haffajee *et al.*, 1997). Thus, the reduction of the levels of these putative periodontal pathogens point out the effectiveness of ultrasonic treatment in deep pockets. The incomplete elimination of periodontal pathogens by non-surgical therapy can be explained by the ability of these bacteria to invade periodontal tissues, and their capacity in evading the host defence, thus causing tissue breakdown.

Certainly, ultrasonic treatment complies with the concept of "critical mass" (WWP, 1989). As applied to non-surgical therapy, the concept of critical mass is best understood by assuming that a major goal of periodontal therapy is to reduce the quantity (mass) of bacterial plaque to a level (critical) that results in a balance between the residual microbes and the host response.

In conclusion, under the conditions of this study, the Vector®-system was unable to eradicate the periodontal pathogens. However, our results indicate that this ultrasonic treatment is able to significantly reduce the quantity of bacterial plaque and to effect a clinically and statistically significant reduction of the periodontal pocket depth. Therefore, the utilization of this ultrasonic device could provide a valid and effective alternative to the conventional therapy of severe periodontitis.

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