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ORIGINAL ARTICLE

Photodynamic therapy as adjunct to non-surgical periodontal treatment in patients on periodontal maintenance: a randomized controlled clinical trial

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Abstract Recent preclinical and clinical data have suggested the potential benefit of photodynamic therapy (PDT) in the treatment of periodontitis. However, currently, there are very limited data from controlled clinical trials evaluating the effect of PDT in the treatment of periodontitis. The aim of the present study was to evaluate the clinical and microbiological effects of the adjunctive use of PDT in non-surgical periodontal treatment in patients receiving supportive periodontal therapy. Twenty-four patients receiving regularly supportive periodontal therapy were randomly treated with either subgingival scaling and root planing followed by a single episode of PDT (test) or subgingival scaling and root planing alone (control). The following parameters were evaluated at baseline and at 3 months and 6 months after therapy: full mouth plaque score (FMPS), full mouth bleeding score (FMBS), bleeding on probing (BOP) at experimental sites, probing pocket depth (PPD), gingival recession (REC), and clinical attachment level (CAL). Primary outcome variables were changes in PPD and CAL. Microbiological evaluation of *Aggregatibacter actinomycetemcomitans* (*A.a.*), *Porphyromonas gingivalis* (*P.g.*), *Prevotella intermedia* (*P.i.*), *Tannerella forsythensis* (*T.f.*), *Treponema denticola* (*T.d.*), *Peptostreptococcus micros* (*P.m.*), *Fusobacterium nucleatum* (*F.n.*), *Campylobacter rectus* (*C.r.*), *Eubacterium nodatum* (E.n.), *Eikenella corrodens* (*E.c.*), and *Capnocytophaga* species (*C.s.*) was also performed at baseline and at 3 months and 6 months after therapy, using a commercially available polymerase chain reaction test. No differences in any of the investigated parameters were observed at baseline between the two groups. At 3 months and 6 months after treatment, there were no statistically significant differences between the groups in terms of PPD, CAL and FMPS. At 3 months and 6 months, a statistically significantly higher improvement of BOP was found in the test group. At 3 months after therapy, the microbiological analysis showed a statistically significant reduction of *F.n.* and *E.n.* in the test group. At 6 months, statistically significantly higher numbers of *E.c.* and *C.s.* were detected in the test group. The additional application of a single episode of PDT to scaling and root planing failed to result in an additional improvement in terms of PPD reduction and CAL gain, but it resulted in significantly higher reduction of bleeding scores than following scaling and root planing alone.

Keywords Photodynamic therapy · Non-surgical periodontal therapy · Patients on periodontal maintenance · Randomized controlled clinical study

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Introduction

Periodontitis is a chronic inflammatory disease involving the supporting structures of the teeth. It is triggered by

periodontopathogens, while the clinical outcome is greatly influenced by the local host immune response [1]. The main approach to treating periodontitis involves the removal of supragingival and subgingival plaque biofilm by means of mechanical debridement (i.e. non-surgical periodontal therapy) [2, 3]. Although, in most cases, non-surgical periodontal therapy results in significant clinical improvements, complete removal of bacterial deposits is still very difficult to be accomplished [4]. In order to additionally facilitate bacterial reduction, especially in cases that did not adequately respond to conventional mechanical treatment, complementary methods such as for example systemic and local antibiotics or various laser systems have been proposed [2, 5, 6].

Photodynamic therapy (PDT) was introduced in medical therapy in 1904 as the light-induced inactivation of cells, microorganisms or molecules and is based on the principle that a photosensitizer (i.e. a photoactivatable substance) binds to the target cells and can be activated by light of a suitable wavelength [7]. Following the activation of the photosensitizer through light of a certain wavelength, singlet oxygen and other very reactive agents are produced that are extremely toxic to certain cells and bacteria [8, 9].

The action mechanism of PDT can be briefly described as follows: upon illumination, the photosensitizer is excited and converted from the ground state to the triplet state. The longer lifetime of the triplet state enables the interaction of the excited photosensitizer with the surrounding molecules, and it is generally accepted that the generation of the cytotoxic species produced during PDT occurs whilst in this state [8]. The cytotoxic product, usually singlet oxygen ($^1O_2$), has a short lifetime in biological systems (<0.04μs) and, therefore, was also shown to have a short radius of action (0.02 μm). Because of the limited migration of the $^1O_2$ from the site of its formation, sites of initial cell damage from PDT are closely related to the localization of the sensitizer, thus making it ideal for local application of PDT, without endangering distant molecules, cells or organs [10, 11]. Data from in vitro studies have shown that several periodontal pathogens, such as *Porphyromonas gingivalis* (*P.g.*) *Prevotella intermedia* (*P.i.*), *Aggregatibacter actinomycetemcomitans* (*A.a.*), *Fusobacterium nucleatum* (*F.n.*), are efficiently eradicated by photodynamic therapy, both in aqueous suspension and as a biofilm, while the presence of substances typical of the oral environment, such as demineralized dentine and collagen, does not interfere with the kinetics and efficiency of the photoprocess [12–15]. Toluidine blue has been shown to be a highly effective photosensitizer when used in combination with a helium/neon (He/Ne) soft laser irradiation [12, 16]. Data from an in vitro study have demonstrated the bactericidal effect of photosensitization with toluidine blue and irradiation with a He/Ne soft laser on different implant surfaces, which resulted in elimination of *A.a.*, *P.g.* and *P.i.* [17]. Results from experimental studies on rats have demonstrated that, in rats affected by ligature-induced periodontitis, toluidine blue–PDT can selectively kill *P.g.* in the oral cavity and significantly decrease the level of alveolar bone loss [15, 18]. Furthermore, findings from an experimental study on dogs have indicated that PDT using a photosensitizer and a 662 nm laser light source may significantly reduce bleeding on probing (BOP) and suppress *P.g.* [19]. Hayek et al. [20] have compared, in a ligature-induced peri-implantitis model in dogs, the effect of PDT, consisting of a paste-based azulene sensitizer followed by irradiation with a gallium–aluminium–arsenium (GaAlAs) diode laser (660 nm), with that of conventional peri-implantitis treatment (i.e. flap surgery and irrigation with chlorhexidine). Both treatments resulted in significant reductions in counts of *Prevotella* sp., *Fusobacterium* sp., and *Streptococcus beta-haemolythicus*; however, there were no significant differences between the two treatments. Positive effects of PDT, using toluidine blue and GaAlAs diode laser (685 nm), upon ligature-induced peri-implantitis in dogs were also reported [21]. Moreover, the use of PDT in conjunction with guided bone regeneration (GBR) in the treatment of peri-implantitis in dogs resulted in better osseointegration than with GBR alone [22].

In a clinical study, Dörtbudak et al. [23] evaluated the effect of PDT in the treatment of peri-implantitis. Fifteen patients with oral implants showing clinical and radiographic signs of peri-implantitis were treated by means of flap surgery and removal of granulation tissue with plastic scalers. Subsequently, the defects were treated as follows: (a) rinsing with saline solution (control group—group 1), (b) application of toluidine blue (group 2) or (c) application of toluidine blue and activation by means a diode laser with a wavelength of 690 nm (group 3). The results showed that the combined treatment (i.e. application of toluidine blue and activation by means a diode laser) yielded the most significant reduction of *A.a.*, *P.g.* and *P.i.* A very recent controlled clinical study has compared, in patients with aggressive periodontitis, the effects PDT treatment alone (i.e. without subgingival scaling and root planing) with subgingival scaling and root planing alone. At 3 months following therapy, both treatments yielded comparable outcomes in terms of reduction of bleeding on probing and probing pocket depths (PPDs) and gains in clinical attachment level (CAL), thus suggesting a potential clinical effect of PDT [24].

However, to the best of our knowledge, data from controlled clinical trials evaluating the effect of PDT are, currently, still limited. Moreover, to the best of our knowledge, there are no available data evaluating the clinical and microbiological effects of the adjunctive use of PDT in non-surgical periodontal treatment on patients on periodontal maintenance.

Therefore, the aim of the present prospective, controlled, clinical study was to evaluate clinically and microbiologi-

cally the effectiveness of the adjunctive use of PDT in non-surgical periodontal treatment in patients on periodontal maintenance.

Materials and methods

Calculation of sample size

The calculation of the sample size determined that ten subjects per treatment group would provide 80% power to detect a true difference of 1.0 mm between test and control, using probing depth (PD) reduction in pockets as the primary outcome variable, assuming that the common standard deviation would be 0.8 mm. Accordingly, a sample of 12 subjects per group (24 in total) were to be recruited to compensate for possible drop-out during the study period.

Subjects and study design

Twenty-four patients suffering from periodontitis and participating in a supportive periodontal therapy programme in the Department of Periodontology at the St. Radboud University Nijmegen Medical Center were recruited. All patients signed an informed consent form, and the study was in accordance with the Helsinki Declaration of 1975, as revised in 1983. The participants had to meet the following inclusion criteria: (a) chronic periodontitis [25], (b) no active periodontal treatment during the past 6 months, (c) presence of at least one sit per quadrant exhibiting pocket depth of ≥4 mm with bleeding on probing, (d) good general health without any signs of systemic disease, (e) no use of antibiotics for the past 12 months and (f) no pregnancy.

The patients were randomly allocated by a toss of a coin [26] to the control or the test group. Each group comprised 12 patients. The baseline characteristics of the patients are shown in Table 1.

Treatment

Prior to study initiation, the therapist performing the periodontal treatment (P.C.) was educated and trained in the use of the laser unit and the ultrasonic device under the instructions and supervision of the manufacturers.

Table 1 Patients' parameters at baseline

Baseline measurements	Control group (n=12)	Test group (n=12)	P
Age in years (mean±SD)	50.6 (±9.2)	48.3 (±7.9)	>0.05
Gender (Female/male)	7/5	7/5	>0.05
Smoking (yes/no)	3/9	4/8	>0.05
Number of sites with PPD ≥ 4 mm on a patient level (mean±SD)	9.9 (±2.9)	10.7 (±3.2)	>0.05

All treatments were performed under local anaesthesia. The patients assigned to the control treatment received mechanical debridement with a sonic scaler (Sonicflex®, KaVo Dental GmbH, Biberach, Germany) with power set to 6000 Hz and water as coolant. The instrumentation was terminated when the operator judged the debridement to be adequately performed. In the test group, patients received mechanical debridement in the same manner as the ones in the control group. Additionally a dye/laser system was applied. The system consisted of a hand-held battery-operated diode laser (HELBO® minilaser 2075 F dent, HELBO Photodynamic Systems GmbH & Co KG, Grieskirchen, Austria). The laser wavelength was 670 nm, and the power density 75 mW/cm^2. The dye was a commercial solution based on a phenothiazine chloride (HELBO Blue Photosensitizer®, HELBO Photodynamic Systems). The photosensitizer was carefully applied with a blunt needle at the bottom of the periodontal pocket in a circular motion. Afterwards, the diode laser unit was used with an 8.5 cm-long flexible tip curved at an angle of 60°. Working time was 60 s per tooth. Only sites with deep pockets (PPD≥4 mm) were treated subgingivally, either with the conventional approach (control group) or the combined approach (test group), and these sites were included in the statistical analysis (experimental sites).

Subsequently, supragingival cleaning was performed with a rubber cup and a low-abrasive polishing paste for both groups. At 3-month and 6-month intervals, the supragingival cleaning was repeated. In addition, self-performed plaque control measures were reinforced when indicated.

Sites with clinical signs of deterioration (CAL≥3 mm) during the experimental period were planned to be excluded from the study and treated with supra/subgingival scaling and root planing under local anaesthesia. Upon completion of the study all subjects received full care and then continued with their individualized maintenance programme.

Clinical assessment

Clinical data were collected before treatment (baseline) and at follow-up examinations after 3 months and 6 months by an examiner (D.N.) not aware of the treatment allocation with respect to treatment assignment. For probing measurements, a manual periodontal probe (UNC-15, Hu-Friedy Co., Chicago, IL, USA) was used.

The variables recorded were as follows:

- Probing pocket depth (PPD) was measured to the nearest millimetre from the gingival margin to the base of the clinical pocket. Only sites with deep pockets (PPD≥4 mm) were included in the statistical analysis (experimental sites).

- Recession of the gingival margin (REC) was recorded to the nearest millimetre from the cemento-enamel junction to the gingival margin, at the experimental sites.
- Clinical attachment level (CAL) was calculated as distance in millimetres from a fixed reference point (cemento-enamel junction or the border of a restoration) to the bottom of the probeable pocket, at the experimental sites.
- Full mouth plaque score (FMPS) was recorded as the percentage of tooth surfaces that revealed the presence of plaque detected by the use of a periodontal probe, modified from O'Leary et al. 1972 [27].
- Full mouth bleeding score (FMBS) was assessed simultaneously with the pocket measurements, based on the presence or absence of bleeding up to 30 s after probing had been recorded. Additionally, bleeding on probing (BOP) was calculated selectively for the experimental sites.

Intra-examiner reproducibility

Five patients, not related to the study, each showing two pairs of contralateral teeth with probing depths >5 mm on at least one aspect of each tooth, were used as calibration for the examiner. The examiner (D.N.) examined the patients on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar, to the millimetre, in more than 90% of the duplicated measurements.

Microbiological evaluation

At baseline, subgingival plaque samples were collected by the therapist (P.C.) from the deepest site in each quadrant, using a commercially available kit (micro-IDent®, Hain Lifescience GmbH, Nehren, Germany). The same sites were tested at 3 months and 6 months. After meticulous removal of supragingival plaque and calculus with sterile periodontal curettes, each selected site was dried and isolated from water and saliva with cotton rolls. Subsequently, the subgingival plaque samples were collected using sterile paper points (#50, Hain Lifescience that were inserted and left in place for 20 s. The paper points from each site were then pooled together in sterile transport vials and sent to a laboratory for bacterial DNA analysis (Microdent, Add Benelux, Malden, The Netherlands).

With a commercially available kit (micro-IDent®, Hain Lifescience) DNA was analysed in the laboratories of Hain Lifescience. The analysis was performed to identify the following micro-organisms: Aggregatibacter actinomycetemcomitans (*A.a.*), *Porphyromonas gingivalis* (*P.g.*), *Prevotella intermedia* (*P.i.*), *Tannerella forsythensis* (*T.f.*), *Treponema denticola* (*T.d.*), *Peptostreptococcus micros* (*P.m.*), *Fusobacterium nucleatum* (*F.n.*), *Campylobacter rectus* (*C.r.*), *Eubacterium nodatum* (E.n.), *Eikenella corrodens* (*E.c.*), and *Capnocytophaga* species (*C.s.*).

The microbiological analysis consisted of:

(a) DNA isolation from the paper points: 400 μl 5% Chelex 100 in 10 mM Tris pH 8.5 were added to every sample. After centrifugation of the paper points, the samples were placed in an ultrasonic bath (Branson 5510) at 60°C for 15 min. Finally, the samples were incubated for 15 min in a 105°C thermo-block. Following centrifugation, 5 μl was used for the polymerase chain reaction (PCR).

(b) PCR technique: 10 μl buffer, 35 μl primer nucleotide mix (multiplex micro-IDent® kit) and 0.2 μl Tag polymerase per sample were premixed, and 5 μl DNA sample solution was added. The amplification profile was one cycle at 95°C/5 min, ten cycles at 95°C/30 s and 58°C/2 min, 20 cycles at 95°C/25 s, 53°C/40 s and 70°C/40 s, one cycle at 70°C/8 min. For the species *Peptostreptococcus* until *Capnocytophaga*, a second primer nucleotide mix was used (multiplex micro-IDent®Plus kit). The PCR procedure was identical to that of the micro-IDent® kit.

(c) Reverse hybridization: for the automatic executed hybridization, an Apollo blot automat (Matec) was used; 20 μl of the amplified sample was mixed with 20 μl of the denaturizing solution, for 5 min at room temperature. One millilitre of pre-warmed hybridization buffer was added. The specific DNA probes for the reverse hybridization were fixed on a membrane strip. Under gentle shaking, the strip was incubated for 30 min at 45°C. After aspiration of the hybridization buffer, 1 ml of stringent wash buffer was added and the strip was incubated for 15 min at 45°C. The strip was washed for 1 min with the rinse solution at room temperature. The conjugate (streptavidin-conjugated alkaline phosphatase) was added, and the strip was incubated for 30 min at room temperature. After being washed twice for 1 min with water, the strip was dried between absorbing papers, and the results were evaluated, quantified and interpreted.

Validated quantitative and qualitative test samples were used as control samples. Bacterial levels were expressed as genome equivalents ($< 10^3$=0, 10^3 to 10^4=1, 10^4 to 10^5=2 and 10^5 to 10^6=3). The test had a detection limit of 10^3 genome equivalents.

Statistical analysis

The statistical analysis was performed with commercially available software (Instat® 2000, version 3.05, GraphPad

Software Inc., San Diego, USA). Primary clinical outcome variables were changes in PPD and CAL. Mean values and standard deviations (mean ± SDs) for the clinical variables were calculated for each treatment, based on the subject as the statistical unit. Student's *t*-test was employed for continuous variables (clinical measurements) after the normality of the data distribution had been confirmed. Likewise, the significance of the difference within each group before and after treatment was evaluated with the paired-samples *t*-test. Ordinal data (microbiological values) were analysed with the Mann–Whitney U test. Differences were considered statistically significant when the P value was less than 0.05.

Results

Clinical assessment

All enrolled patients completed the 6-month study, and there were no sites showing clinical signs of deterioration. Healing was uneventful in all patients. Neither pain nor any other discomfort was reported by any of the patients following both treatments.

The clinical measurements depicting the treated sites at baseline and at 3 months and 6 months are shown in Table 2. At baseline, there were no significant differences between test and control in any of the parameters evaluated. Changes in PPD and CAL showed in both groups a statistically significant difference between baseline and 3 months and between baseline and 6 months. In the test group mean PPD change measured 0.6±0.6 mm (P=0.004) at 3 months and 0.8±0.5 mm (P=0.001) at 6 months. The corresponding values for CAL change were 0.5±0.4 mm (P=0.001) and 0.7±0.7 mm (P=0.005), respectively. In the control group mean PPD changes at 3 months and 6 months measured 0.6±0.5 mm (P=0.002) and 0.9±0.8 mm (P=0.002), respectively. The corresponding values for CAL change were 0.4±0.5 mm (P=0.009) and 0.5±0.6 mm (P=0.014).

No statistically significant differences in terms of plaque scores were observed between the two groups at any time point (Table 3). For full mouth bleeding score (FMBS), there was a significant difference between the groups at 6 months examination in favour of the test group (Table 3). The BOP values at experimental (i.e. treated) sites indicated a significant effect in favour of the test group (Table 4).

Microbiological evaluation

The prevalence and difference in bacterial levels of each bacterial species analysed between the groups are presented in Figs. 1, 2 and 3. Considering only the presence of bacteria belonging to the "red complex" (*P.g.*, *T.f.*, *T.d.*), "orange complex" *(P.i.*, *P.n.*, *F.n.*, *P.m.*, *C.r.*) and "green complex" (*E.n.*, *E.c.* and *Capnocytophaga* species) as defined by Socransky et al. [28], we detected a significant effect for *T.d.*, *F.n.*, *E.n.*, *E.c.* and *Capnocytophaga* species. For the test group there was a statistical significant reduction at 3 months for *F.n.* and *E.n.* (Fig. 2) and at the 6-month evaluation for *E.c.* and *C.s.* (Fig. 3) in comparison with the control group findings for the same time intervals. Interestingly, there was also a significant difference in the prevalence of *T.d.* between the two study groups at the 6-month evaluation (Fig. 3).

Discussion

Our study evaluated the clinical and microbiological effects of the adjunctive effect of PDT in non-surgical periodontal

Table 2 Clinical assessment of CAL, PPD and REC at the experimental (i.e. treated) sites

Parameter	Baseline	At 3 months	At 6 months	Difference 0–3 months	*P*	Difference 0–6 months	*P*
PPD (mm)							
Test	5.2±0.4	4.6±0.7	4.4±0.8	0.6±0.6	0.004	0.8±0.5	0.001
Control	5.6±0.7	5.0±0.7	4.7±0.9	0.6±0.5	0.002	0.9±0.8	0.002
P				0.851		0.877	
REC (mm)							
Test	1.5±0.9	1.7±0.9	1.7±0.9	0.2±0.4	0.280	0.2±0.4	0.189
Control	1.5±0.6	1.7±0.6	1.9±0.6	0.2±0.3	0.214	0.4±0.3	0.001
P				0.897		0.073	
CAL (mm)							
Test	6.8±0.9	6.3±0.9	6.1±0.9	0.5±0.4	0.001	0.7±0.7	0.005
Control	7.1±0.9	6.7±0.9	6.6±0.9	0.4±0.5	0.009	0.5±0.6	0.014
P				0.722		0.572	

Table 3 Full mouth plaque and bleeding scores

Parameter	Control group	Test group	*P*
FMPS (percentage)			
Baseline	33±19	22±10	0.083
At 3 months	22±11	16±06	0.116
At 6 months	25±17	18±10	0.271
FMBP (percentage)			
Baseline	19±14	15±12	0.454
At 3 months	14±08	11±04	0.381
At 6 months	18±08	12±05	0.041

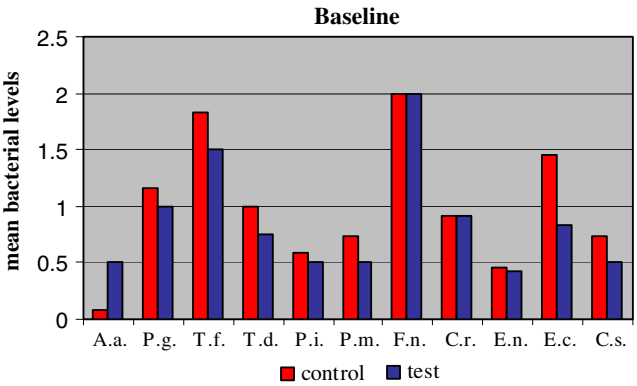

Fig. 1 Mean levels of tested bacteria categorized by treatment at baseline

treatment in patients on periodontal maintenance. Compared with baseline and at 3 months and 6 months, both treatments resulted in statistically significant PPD reduction and CAL gain. No statistically significant differences in terms of PPD and CAL changes were found between the treatments. These clinical results compare well with those of a recent controlled clinical study evaluating the effects of non-surgical periodontal therapy in patients on maintenance programmes treated with either an erbium:yttrium–aluminium–garnet (Er:YAG) laser or an ultrasonic device [29]. In that study, at 4-months after therapy, treatment with Er:YAG laser had resulted in a mean PPD reduction of 1.1 mm and a mean CAL gain of 0.6 mm, while the ultrasonic instrumentation yielded a mean PPD reduction of 1.0 mm and a mean CAL gain of 0.4 mm. The results failed to demonstrate any statistically significant differences between the two treatments in the clinical parameters investigated [29].

On the other hand, the results also showed that mechanical debridement followed by additional application of PDT resulted, at both 3 months and 6 months, in statistically significant higher BOP and FMBS reductions than from mechanical debridement alone. This observation is in agreement with the findings of a recent meta-analysis of preclinical and clinical studies, which indicated that low-level laser therapy (based also on a photodynamic effect) may positively influence several processes associated with tissue repair, such as acceleration of inflammation, wound tensile strength, reduction of wound size, healing time and collagen synthesis [30]. Furthermore, in a recent controlled clinical trial, a beneficial effect upon gingival inflammation, indicated by a significant decrease in the volume of gingival crevicular fluid (GCF), was also demonstrated after low-level laser had been used as adjunct therapy in the treatment of periodontal inflammation [31].

When the clinical and microbiological effects obtained with PDT are being interpreted, the possible effects due to the application of the photosensitizer itself need to be pointed out. Dörtbudak et al. [23] reported that treatment of peri-implantitis with application of the photosensitizer toluidine blue alone (i.e. without light sensitization) resulted in significant reductions of *P.i.* and *A.a.* compared to baseline values. The bacterial counts of *P.g.* also decreased in comparison with the initial value, but the change was not statistically significant. On the other hand, the lethal photosensitization of the toluidine blue with a diode laser of a wavelength of 690 nm resulted in significantly higher reductions of *P.i.*, *A.a.*, and *P.g.* compared to both baseline

Table 4 Bleeding score at the experimental (i.e. treated) sites

Parameter	Control group	Test group	*P*
BOP (percentage)			
Baseline	58±34	69±26	0.413
At 3 months	50±26	17±19	0.002
At 6 months	48±36	19±22	0.027

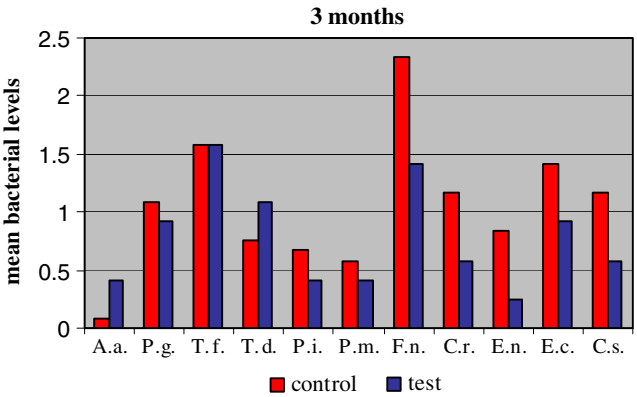

Fig. 2 Mean levels of tested bacteria categorized by treatment at 3 months

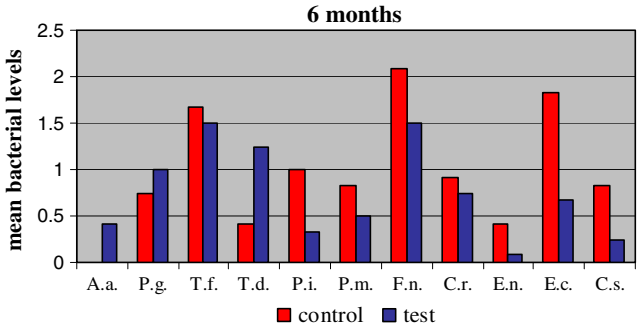

Fig. 3 Mean levels of tested bacteria categorized by treatment at 6 months

and application of toluidine blue alone. It has, however, to be kept in mind that complete elimination of these bacteria was not achieved in any of the cases [23]. These results are in accordance with previous findings evaluating the use of photodynamic therapy on oral bacteria which have shown that the combination of a photosensitizer with low-power laser irradiation was effective in killing *A.a.*, *P.g.* and *F.n.* [32]. Moreover, it was also demonstrated that complete elimination of *A.a.*, *P.g.* and *F.n.* was also possible if photodynamic therapy was used against bacteria organized in biofilms [12].

In our study, both treatments resulted in significant reductions in *T.d.*, *F.n.*, *E.n.*, *E.c.* and *Capnocytophaga* species. The only differences between the two treatments were statistically significant reductions of *F.n.* and *E.n.* at 3 months and *E.c.* and *C.s.* at 6 months after treatment with PDT. These differences are, currently, difficult to explain. Furthermore, direct comparisons of the present microbiological findings with those reported by others following PDT are also difficult. It is well known that the results of in vitro or animal studies cannot always be directly extrapolated to the human situation, and, therefore, they need to be interpreted with caution [33, 34]. Moreover, the available clinical studies have evaluated the effect of PDT in surgical treatment of peri-implantitis and not in conjunction with non-surgical therapy in patients on periodontal maintenance [23].

Another interesting finding was the *T.d.* increase in the test group. This finding is very difficult to interpret, since none of the available studies has evaluated changes of *T.d.* levels and, currently, there are no other studies to compare with [12, 23, 32].

A question which may arise when photodynamic therapy is used is the possible toxic effects related to the photosensitizer. In our study the photosensitizer was phenothiazine chloride, at a concentration of 10 mg/ml. The main component of phenothiazine chloride is toluidine-blue, a sensitizer which has been extensively tested in in vitro, preclinical and clinical studies [12–15, 23, 24]. In none of these studies were any toxic effects related to the photosensitizer reported. The photosensitizer is Food and Drug Administration (FDA) approved and was also used in previous clinical studies [23, 24]. Also, the application time (1 min) for the photosensitizer and the wavelength of the diode laser (690 nm) were exactly the same as those in the studies mentioned [23, 24]. Thus, our results, together with the available data, strongly indicate that the photosensitizer used does not exhibit any systemic or local toxic effects.

The lack of other data from controlled clinical studies evaluating the effect of non-surgical periodontal therapy with PDT makes direct comparisons impossible. In a very recent controlled clinical study of patients with aggressive periodontitis, treatment with PDT alone (i.e. without subgingival scaling and root planing) was compared with subgingival scaling and root planing (SRP) [24]. At 3 months following therapy, the mean PPD decreased in the PDT group from 4.92±1.61 mm to 3.49±0.98 mm and in the SRP group from 4.92±1.14 mm at baseline to 3.98±1.76 mm. The mean relative clinical attachment (RCAL) decreased in the PDT group from 9.93±2.10 mm at baseline to 8.74±2.12 mm and in the SRP group from 10.53±2.30 mm at baseline to 9.01±3.05 mm. There were no statistically significant differences in any of the clinical parameters investigated, thus indicating similar results of the two treatments in the non-surgical treatment of aggressive periodontitis [24].

In another review, it was suggested that PDT may still bear some possible benefits, such as an additional effect at sites with difficult access (e.g. furcations, deep invaginations, concavities), influencing the biofilm in residual deep pockets, decreasing the risk of bacteraemia which routinely occurs after periodontal treatment or as an alternative for diminishing the danger of an increase in antibiotic resistance (Meisel and Kocher 2005 [35]). On the other hand, it needs to be kept in mind that, currently, there are no other data from controlled clinical studies comparing PDT used in conjunction with non-surgical periodontal therapy with PDT alone, SRP alone, or the photosensitizer alone (i.e. used without light activation). Such studies are warranted before any definitive conclusions on the possible clinical benefit of PDT used in conjunction with non-surgical therapy can be drawn.

In conclusion, within its limits, our study showed that the additional application of a single episode of PDT to scaling and root planing failed to result in additional improvement in terms of PPD reduction and CAL gain, but it resulted in significantly higher reduction of bleeding scores than with scaling and root planing alone.

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