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Research Article

Non-Surgical Therapy for Chronic Periodontitis: Adjunctive Photochemical Therapy for Accentuated Outcomes?

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Abstract

Background and Objectives: The main objective of periodontal treatment is to control infection and thereby curb disease progression. Photodynamic therapy provides a broad spectrum of antimicrobial activity without any local or systemic side effects. The aim of the present study is to evaluate the efficacy of a new photosensitizer (indocyanine green), which has high absorption at a wavelength of 800 - 810 nm for its photodynamic potential. A split mouth randomized controlled clinical trial was planned to compare the clinical and microbiological outcomes of scaling and root planing with photodynamic therapy as compared to scaling and root planning alone.

Method: In a split mouth study, 10 patients presenting with at least two teeth on contra lateral sides having at least one site with a probing pocket depth of greater than 5 mm were selected for the study. Control sites received only scaling and root planning whereas in test sites scaling and root and root planing was followed by a single session of photodynamic therapy. Plaque index, gingival index, sulcular bleeding index, probing pocket depth and relative attachment level were measured at baseline, 1 month and 3 months. Microbiologic evaluation was performed using anaerobic colony count test at baseline and 3 months.

Results: At 1 and 3 months after treatment, there were no statistically significant differences between the groups with regard to probing pocket depth, relative attachment level, plaque index, or microbiologic changes. However, a greater improvement in gingival index and sulcular bleeding index was found in the test group at 1 and 3 months which was statistically significant.

Conclusions: The additional application of a single episode of photodynamic therapy to scaling and root planing failed to result in an additional improvement in terms of pocket depth reduction and relative attachment level gain, but it did result in a significantly higher reduction in gingival inflammation and bleeding scores compared to scaling and root planing alone.

Keywords: Chronic Periodontitis; Split Mouth Design; Scaling and Root Planing; Photosensitizer; Laser; Photodynamic Therapy

Introduction

The mainstay of periodontal therapy inspite of various advances in therapeutic regimens is non-surgical therapy in form of scaling and root planning. It is an established fact that plaque is nothing but a microbial biofilm with its own inpenetrability to antimicrobial agents. A routine scaling and root planning has its own limitations in the form of anatomical barriers like complex root structure, furcation areas, root concavities, deep developmental grooves etc [1]. It is all the more impossible as the pockets gets deeper. The current principles of periodontal treatment involve a combination of periodic mechanical disruption of dental plaque with use of adjunctive antimicrobial agents. The anti-infective therapy can be systemic or local. The systemic route of administration of antibiotics has its own disadvantages mainly insufficient concentration in gingival sulcus, development of bacterial resistance and individual adverse drug reactions [2]. Although Local drug delivery can overcome some of these disadvantages. It is still not efficient and lacks evidence.

Photodynamic therapy is also known as known as photo radiation therapy, phototherapy or photo chemotherapy has its origin in the early 1900 s due to the discovery by Raab of an interaction between acridine and visible light in the presence of having the capability to kill paramecia. The biggest perk of Photodynamic therapy is its non-invasiveness. There are mainly two components to photodisinfection i.e. laser light and photosensitizer [3]. A photosensitizer has the unique ability to absorb light of a specific wavelength and transforming it into useful energy. Photodynamic therapy has bactericidal effect by mechanism of DNA damage and disrupting the cytoplasmic membrane of the bacteria. There is also some regulation of the reaction by controlling the amount of light that can be applied to activate the dye. Thus, the aim of the present study was to observe the additional benefits rendered to non-surgical therapy by photodisinfection.

Materials and Methods

Source of Data

The subjects screened for chronic periodontitis and referred for periodontal treatment at the Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore were included in the study after having signed an informed consent. A total of 10 chronic periodontitis subjects, with at least two teeth on contra lateral sides having at least one site with a probing pocket depth of ≥ 5 mm, and presence of bleeding on probing (BOP) were included in the study.

Selection Criteria

Inclusion criteria:

- Patients within the age group of 18 - 65 years [4].
- Patients with periodontal pockets on contra lateral side with probing depth of ≥ 5 mm.
- Patients exhibiting a plaque index of < 30% upon completion of the initial oral hygiene programme [5].
- Systemically healthy subjects [4].
- Patients willing to participate in the study.

Exclusion criteria

- Patients who have received any periodontal therapy (non-surgical or surgical) 6 months prior to the initial examination [5].
- Patients who have consumed antibiotics within the last 6 months [5].
- Medically compromised patients [5].
- Pregnant and lactating women.
- Smokers (both present and past) [5]
- Subjects allergic to photosensitizer [5].

The present study was a 3 months parallel, single-center, placebo controlled randomized clinical trial using a single time application of Photodynamic Therapy as an adjunct to nonsurgical periodontal therapy (NSPT) in patients with chronic periodontitis. At the beginning of the study, two types of therapy were selected: scaling and root planing (SRP) or SRP and photodynamic therapy (SRP + PDT). The treatment was done according to a-split mouth design. The sites selected were divided into:

- **Test site:** Treatment with scaling and root planning followed by single application of photodynamic therapy and microbiologic assessment at baseline and 12 weeks.
- **Control site:** Treatment with only scaling and root planning along with microbiologic assessment at baseline and after 12 weeks.

Clinical parameters

The periodontal status of each subject was assessed at baseline, 1 month and 3 months after periodontal treatment. Following clinical parameters were recorded:

- Plaque index [6].
- Gingival index [7].
- Modified sulcular bleeding index [8].
- Periodontal probing depth.
- Relative attachment levels.

Microbiological analysis

Subgingival plaque samples were taken at baseline from the deepest pocket per quadrant.

The same site was resampled at 12 weeks.

Following meticulous removal of supra gingival calculus and plaque using sterile standard periodontal scalers and sterile cotton pellets, each selected site was dried and isolated from water and saliva using cotton rolls. Subsequently, a sterile paper point (size 30) was inserted and left in place for 30 seconds and later was transferred to 10 ml of sodium thioglycolate media and subsequently incubated for two hours. The samples were then serially diluted and 10 microlitre of diluted specimen was streaked onto blood agar supplemented with hemin (5 mg/ml) and vitamin k (10 mg/ml) and anaerobically cultured using anaerobic jar at 35 - 37ºC for 2 - 3 days. All samples were finally inspected for total anaerobic colony count using the digital colony counter. The presumptive identification of various bacteria was made on the basis of colony morphology, gram staining reaction and aero-tolerance.

Procedure

All subjects were treated within 24 hours with SRP using hand instruments and ultrasonic instrumentation followed by a single episode of PDT (test) or SRP using hand instruments and ultrasonic instrumentation (control). Oral hygiene instruction individualized for every subject was given at the first appointment followed by initial periodontal treatment. Following local anesthesia, subgingival instrumentation for test and control groups was performed by employing both hand instruments (Gracey curettes, Hu-Friedy, Chicago, IL, USA) and piezo electric ultrasonic instruments (EMS - Piezon® 250) until the operator believed that the root surfaces were adequately debrided and planed. Randomization was performed immediately following the completion of instrumentation. Each experimental site was randomly assigned to one of the two treatment regimens. Allocation concealment was performed by opaque sealed envelopes. Full-mouth supra- and subgingival debridement was performed. No other treatment was given in control group. In the test group, the photosensitizer liquid (Emundo®) was applied with a blunt needle to the instrumented sites, starting from the apical end of the pocket and moving coronally to avoid entrapment of air bubbles. Three minutes later all pockets were thoroughly rinsed with sterile saline to remove the excess photosensitizer. Immediately after rinsing, irradiation with the diode laser (A.R.C Laser GmbH, Nürnberg, Germany), 810 nm wavelength and 100 mW of power output in continuous mode was carried out. The device used here was a safety class II laser. The pocket was exposed to the laser light using the fiber optic applicator with an illuminator tip for one minute in the same mesiodistal direction. A slow gentle movement of the tip was ensured in both apicocoronal and lateral direction without allowing the tip to rise above the gingival crest.

Laser application was performed circumferentially at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual). Special safety glasses were provided to the patients, operator and dental assistant to prevent possible eye damage by the laser irradiation. In all the cases, treatment was performed by the same experienced operator in a single session under local anesthesia (if required during subgingival instrumentation). The subjects returned at 1month and 3 months for clinical parameters and microbiological evaluation.

The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analysed using SPSS (Statistical Package for Social Science, Ver.10.0.5) package, The student t-test was used to determine whether there was a statistical difference between treatment groups in the parameters measured One way analyses of variance were used to test the difference between groups. Analysis of Variance is a technique by which the total variation is split into two parts one between groups and the other within the groups. If F' value is significant there is a significant, difference between group means. To find out which of the two groups means is significantly difference post hoc test of Tukey test is used.

Results

Figure 1: *Placement of photosensitizer at test site.*

Figure 2A: *Using bleaching handpiece.*

Figure 2B: *Using a bare fiber.*

Figure 2: *Irradiation with laser at test site.*

Figure 3: *Armamentarium used for the study.*

Figure 4A

Figure 4B

Figure 4: *Inoculation and streaking of sample.*

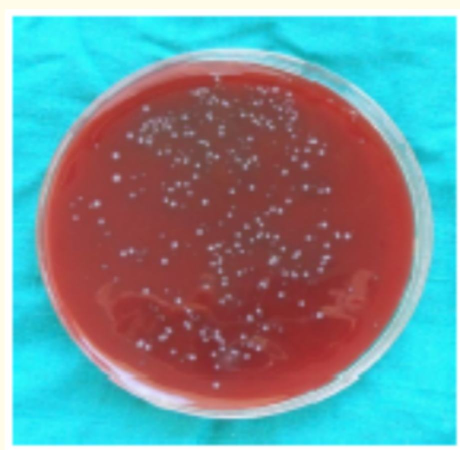

Figure 5A: *At baseline (pre-treatment).*

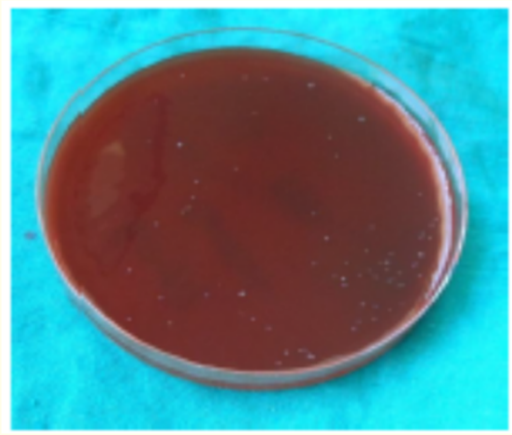

Figure 5B: *3 months after treatment with photodynamic therapy.*

Figure 5: *Anaerobic growth.*

Bleeding index

Test site: The reduction in mean bleeding index score from baseline to 1 month was 2.40 (p < 0.001) whereas from baseline to 3 month, the bleeding index reduced by 2.20 (p < 0.001) showing statistically significant reduction. However, bleeding index increased by 0.200 from 1 month to 3 month which was not statistically significant (p = 0.646).

Contro site: The reduction in mean bleeding index score from baseline to 1 month was 1.300 (p = 0.005) whereas from baseline to 3 month, the bleeding index reduced by 1.100 (p = 0.018) showing statistically significant reduction. However, bleeding index increased by 0.200 from 1 month to 3 month which was not statistically significant (p = 0.856).

	Group	N	Mean	SD	Min.	Max.	't' value	'p' value
Plaque Index	Test	10	1.73	0.392	1.20	2.20	0.161	0.693
	Control	10	1.66	0.389	1.00	2.20		
Gingival Index	Test	10	1.31	0.260	1.00	1.70	7.245	0.015
	Control	10	1.67	0.333	1.20	2.20		
Bleeding Index	Test	10	0.50	0.527	.00	1.00	10.000	0.005
	Control	10	1.50	0.850	.00	3.00		
Pocket Depth	Test	10	3.61	0.905	2.50	5.20	0.107	0.747
	Control	10	3.75	1.005	2.30	4.80		
PAL	Test	10	6.59	1.030	5.00	5.30	1.059	0.317
	Control	10	7.09	1.140	5.50	9.10		

Table 1: *Comparison of clinical parameters between test and control group by student „t" test at 3 month interval.*

	Visit	1 Month		3 Month	
		Mean Diff	'p' value	Mean Diff	'p' value
Plaque Index	Baseline	0.980	< 0.001	0.940	< 0.001
	1 Month	-	-	-0.040	0.966
Gingival Index	Baseline	1.060	<0.001	0.900	< 0.001
	1 Month	-	-	-0.160	0.401
Bleeding Index	Baseline	1.300	0.005	1.100	0.018
	1 Month	-		-0.200	0.856
Pocket Depth	Baseline	0.760	0.258	1.040	0.088
	1 Month	-	-	0.280	0.824
PAL	Baseline	0.580	0.490	0.940	0.167
	1 Month	-	-	0.360	0.756

Table 2: *Intragroup (CONTROL) pairwise comparison of clinical parameters between the visits using Tukey Test.*

	Visit	N	Mean	SD	Min.	Max.	'F' value	'p' value
Plaque Index	Baseline	10	2.60	0.316	2.00	3.00	24.003	< 0.001
	1 Month	10	1.62	0.365	1.20	2.20		
	3 Month	10	1.66	0.389	1.00	2.20		
Gingival Index	Baseline	10	2.57	0.245	2.20	3.00	43.841	< 0.001
	1 Month	10	1.51	0.225	1.20	2.00		
	3 Month	10	1.67	0.333	1.20	2.20		
Bleeding Index	Baseline	10	2.60	0.516	2.00	3.00	6.963	0.004
	1 Month	10	1.30	1.059	0.00	3.00		
	3 Month	10	1.50	0.850	0.00	3.00		
Pocket Depth	Baseline	10	4.79	1.159	3.30	7.00	2.607	0.092
	1 Month	10	4.03	0.990	2.50	5.30		
	3 Month	10	3.75	1.005	2.30	4.80		
RAL	Baseline	10	8.03	1.150	6.50	10.10	1.780	0.188
	1 Month	10	7.45	1.081	5.80	9.30		
	3 Month	10	7.09	1.139	5.50	9.10		

***Table 3**: Comparison of clinical parameters within control group at different recall intervals by One Way ANOVA test.*

Visit	Group	N	Mean	SD	Min.	Max.	't' value	'p' value
Baseline	Test	10	43.4	10.502	29.7	60.8	0.722	0.407
	Control	10	40.1	6.292	32.8	52.9		
3 Month	Test	10	26.3	8.089	16.8	40.9	1.773	0.200
	Control	10	30.6	6.155	21.8	39.8		

***Table 4:** Comparison of change in anaerobic colony count (in million cfu) between test and control group at baseline and 3 months by student t‟ test.*

Gingival index

Test site: The reduction in mean gingival index score from baseline to 1 month was 1.230 (p < 0.001) whereas from baseline to 3 month, the gingival index reduced by 1.140 (p < 0.001) showing statistically significant reduction. However, gingival index increased by 0.090 from 1 month to 3 month which was not statistically significant (p = 0.653).

Control site: The reduction in mean gingival index score from baseline to 1 month was 1.060 (p < 0.001) whereas from baseline to 3 month, the gingival index reduced by 0.900 (p < 0.001) showing statistically significant reduction. However, gingival index increased by 0.160 from 1 month to 3 month which was not statistically significant (p = 0.401).

At 1 and 3 months after treatment, there were no statistically significant differences between the groups with regard to probing pocket depth, relative attachment level, plaque index, or microbiologic changes.

However, a greater improvement in gingival index and sulcular bleeding index was found in the test group at 1 and 3 months which was statistically significant.

Discussion

Bacterial flora within the periodontal pocket exists in a complex heterogeneous biofilm that varies from individual to individual in both the composition and the proportions present [9]. The resistant nature of periodontal bacteria in the biofilm emphasizes the necessity of thorough physical removal of subgingival plaque, along with plaque retention sites. Conventional nonsurgical periodontal therapy which includes mechanical plaque control, scaling and root planing, is the first recommended step and is an indispensable phase of periodontal therapy. Scaling and root planing decreases Gram-negative organisms and increases Gram-positive rods and coccal species, a microbial shift associated with periodontal health, resulting in significant clinical improvements and varying success rates. Photodynamic therapy, a novel treatment procedure, combines the use of a photosensitizer with laser light energy to produce either free radicals or singlet oxygen molecules, which have a cytotoxic effect against periodontopathogens. PDT is mediated by singlet oxygen, which has a direct effect on extracellular molecules, thus, the polysaccharides present in the extracellular matrix of polymers of a bacterial biofilm are also susceptible to photodamage. Such dual activity is not exhibited by antibiotics and may represent a significant advantage of aPDT which is equally effective against antibiotic-resistant and antibiotic-susceptible bacteria. Moreover, development of resistance to the cytotoxic action of singlet oxygen or free radicals seems to be unlikely clearing the way for repeated photosensitization as well. The cultivable microflora in chronic periodontitis lesions comprises about 75% gram negative and 90% anaerobic organisms [10]. Since majority of bacterial species can be grown in cultures, and bacterial cultivation is considered as gold standard, the method of anaerobic culturing was used in the study. Sampling of subgingival plaque was done using paper points because the paper point technique has been proposed to be suitable especially for non-adherent subgingival plaque, and provides acceptable reproducibility, especially for *A. actinomycetemcomitans* and *P. gingivalis.*

Recent literature [11-13] has demonstrated statistically significant improvements in clinical parameters (probing depth and relative clinical attachment level) in sites treated with adjunctive PDT, however the additional gain in CAL and PPD reduction were relatively minimal (0.2 - 0.6 mm). As a reduction of 1 - 3 mm of probing pocket depth could routinely be accomplished with scaling and root debridement It should be noted that in the present study, the range of mean values of baseline PPD in different groups was from 4.7 mm to 5.0 mm. At 3 months, both the treatment groups showed improvements compared to baseline and the mean values of PPD was reduced to 3.6 to 3.7 mm. Since both the treatment groups received SRP, the effectiveness of SRP alone may have masked the adjunctive additive benefits of PDT.

The baseline assessment of periodontal pathogens in the group receiving SRP+PDT and SRP alone was found to be similar. There was a significant reduction in bacterial load in test group from $43.4 \pm 10.5 \times 10^6$ to $26.3 \pm 8.0 \times 10^6$ ($p = 0.001$). Even the control group showed a reduced score of bacterial colony count from $40.1 \pm 6.2 \times 10^6$ to $30.6 \pm 6.1 \times 10^6$ which was not negligible. However, on comparison of both the groups, the difference in bacterial load reduction was not statistically significant ($p = 0.200$). These microbiological changes are in comparison with the study by Theodoro LH., *et a*l. [15] in 2011 who noticed significant reduction in the proportion of sites positive for periodontopathogens at 60, 90 and 180 days compared to baseline in both SRP and SRP+PDT sites, however reduction after 180 days was statistically significant in PDT group which was not observed in this current study. This has been validated by Polansky., *et al*. in 2009 [5] who also could not find any statistically significant differences in reduction of periodontal pathogens like *P. gingivalis, Tannerella forsythia* and *Treponema denticola* at baseline as well as 10, 42 and 90 days after treatment in both the PDT+SRP and SRP alone groups. But both of the above mentioned studies used PCR for microbiological analysis.

In the present study, the mean microbial levels decreased significantly in both the groups. These results were very difficult to interpret because there are very few similar clinical studies to compare them to. Conversely, an *in vitr*o study evaluating the use of PDT on oral bacteria showed that the combination of a photosensitizer with low-power laser irradiation was effective in killing Aa, Pg, and Fn. In a similar *in vitro* study, complete elimination of Aa, Pg, and Fn was also possible if PDT was used against bacteria organized in biofilms. However, it is well known that the results of *in vitro* studies cannot always be directly extrapolated to the human situation; therefore, they need to be interpreted with caution. Fontana., *et al*. in 2009 [16] observed that the effect of PAD on the viability of microorganisms is decreased up to 50% in biofilm bacteria in comparison with planktonic culture. Moreover, the presence of serum-derived gingival crevicular fluid and blood in the periodontal pocket could dramatically reduce the efficacy of PAD in clinical situations [14]. In addition, several factors including photosensitizer type and concentration, period of maintenance of drug within the tissue, time for biological response, the pH of the target site, the presence of exudates, the mode of photosensitizer application, the availability of oxygen and the irradiation parameters could also influence the biological response to PAD. There is some ambiguity regarding the number of sessions of PDT. Following the commonly established protocol for frequency of PDT application (once after SRP), which was the case in studies by Christodoulides., *et al*. [17], Chondros., *et al*. [18], Ge., *et al.* [19] even in the present study, single application of PDT was done. Although multiple course of PDT may improve healing outcomes, there is not enough evidence in the form of literature to support the same. This may have partially contributed to the absence of additive effects in test group. Thus the present study using single application of PDT as adjunct to SRP failed to show statistically significant improvement in key clinical parameters of probing pocket depth and relative clinical attachment levels. This however cannot be used to overrule the positive role of PDT, as the secondary clinical parameters namely bleeding score and gingival inflammation did show a drastic decline. In the area of microbiological investigations, though the intra-group differences were statistically significant, the intergroup variability did not amount to much.

On the basis of meta-analysis that of Sgolastra., *et al.* in 2013 [20] observed that aPDT as adjunct treatment to SRP of chronic periodontitis has a modest but significant effect of 0.21 mm PD reduction and 0.36 mm CAL gain at 3-month follow-up than at 6-month follow-up. In this study, at 1 month follow up, pocket probing depth was reduced to 4.06 ± 0.991 in test group and 4.03 ± 0.990 in control group whereas at 3 months, mean pocket probing depth recorded was 3.61 ± 0.905 for test group and 3.75 ± 1.005 for the control group. At 1 month follow up, relative attachment level recorded was 7.31 ± 0.941 in test group and 7.45 ± 1.081 in control group and at 3 months, it was 6.59 ± 1.030 for test group and 7.09 ± 1.140 for the control group.

Chambrone., *et al.* in 2017 [21], Mills., *et al.* in 2017 [22] in the consensus stated that when laser treatment is used as an adjunct to mechanical treatment, current evidence suggested similar or slightly better clinical outcomes compared with laser treatment alone. Current evidence fails to demonstrate a beneficial long-term (> 48 months) effect of laser treatment used as an adjunctive therapy to non-surgical treatment in providing a more maintainable environment. Mills., *et al.* in 2017 [22] also stated that antimicrobial photodynamic therapy is laser treatment used in conjunction with a photosensitizer intended to reduce periodontal pathogenic bacteria. Current evidence supports that aPDT may provide improvements in probing depth and clinical attachment level compared with conventional periodontal therapy for patients with periodontitis or peri-implantitis. However, comparative differences in clinical outcome are modest (< 1 mm) and the clinical significance is open to question.

Taking the above findings into consideration, further researches addressing the specific areas of concern are warranted.

Conclusions

The following conclusions can be drawn from the study:

- With respect to bleeding on probing and gingival index, combined therapy (SRP+PDT) showed statistically significant reduction in both of these parameters as compared to SRP alone at both one month and three month recall intervals.
- In both the SRP and SRP+PDT group, PPD reduction and RAL gain were significantly improved from baseline to three months with SRP+PDT sites showing better outcomes as compared to SRP only sites. But when comparison between both the groups were made, the difference was not statistically significant.
- Total anaerobic colony count showed statistically significant reduction for both test and control sites when compared at baseline and at 3 months with test group showing superior reduction as compared to control group however when intergroup comparison was made, no statistically significant differences were observed at the end of three months.

Thus within the limits of the present study, it can be concluded that the addition of a single episode of PDT to SRP failed to result in statistically significant improvement in terms of PD reduction and CAL gain, but it resulted in a significantly greater reduction in bleeding scores and gingival inflammation compared to SRP alone. For the microbial parameters, no significant differences were found between the test and the control group. Since, the sites treated with PDT healed uneventfully without any adverse effects, PDT can be suggested to be a safer and effective antimicrobial treatment adjunct.

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