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A Chairside Assessment of Antimicrobial Photodynamic Therapy andlaser therapy in the treatment of periodontitis: A clinical Study

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Abstract

Aim: The purpose of the study was to evaluate the efficacy of photodynamic therapy (PDT) and laser therapy as an adjunct to scaling and root planing in the treatment of periodontal pockets.

Method: Thirty sites in ten individuals with probing pocket depth ≥ 5 mm were randomly divided into three groups, Group A (sites treated with scaling and root planning followed by laser therapy), Group B (sites treated with scaling and root planning followed by photodynamic therapy) and control was set as Group C (sites treated with scaling and root planning). Microbiological parameters (using chair side test kit- Benzoyl-DL-arginine-B-napthylamide; BANA test) and clinical parameters (relative attachment level, probing pocket depth, plaque index, gingival index and sulcus bleeding index) were assessed at baseline and at two months.

Results: Group A and Group B sites showed statistically significant improvement (≤ 0.005) in probing pocket depth, relative attachment level, plaque index, gingival index and sulcus bleeding index, whereas, Group C showed statistically significant improvement in plaque index and gingival index only. In case of microbiological parameter, a significant increase in BANA-negative sites was observed in Group A and Group B.

Conclusion: The overall observations in the present study showed promising results with significant reduction in clinical parameters as well as BANA positive sites treated with either laser therapy or photodynamic therapy in comparison to sites treated with scaling and root planing alone. Hence, both the treatment modalities could be a beneficial adjunct to scaling and root planing.

Keywords: *photodynamic therapy, laser therapy, BANA chairside test kit, periodontitis.*

1. Introduction

Microbial biofilms in the oral cavity are involved in the etiology of various oral conditions, including dental caries, periodontal and endodontic diseases, oral malodor, denture stomatitis, candidiasis and dental implant failures. It is generally recognized that the growth of bacteria in biofilms impart a substantial decrease in susceptibility to antimicrobial agents compared with cultures grown in suspension. Current treatment techniques involve either periodic mechanical disruption of oral microbial biofilms or maintaining therapeutic concentrations of antimicrobials in the oral cavity, both of which are fraught with limitations as over-use of antibiotics have been a major culprit in the production of drug-resistant organisms. The development of alternative antibacterial therapeutic strategies therefore becomes important in the evolution of methods to control microbial growth in the oral cavity.[1] One such approach is photodynamic therapy (PDT), also known as photoradiation therapy, phototherapy or photochemotherapy.[2] It was introduced in medical field in 1904 as the light-induced inactivation of cells, microorganisms, or molecules. Use of photoactivable compounds or photosensitizers to cause

photo-destruction of oral bacteria has been demonstrated, indicating that PDT could be a useful adjuvant to mechanical means in eliminating periopathogenic bacteria. PDT involves the combination of visible light, usually through the use of a diode laser and a photosensitizer. The photosensitizer is a compound that is capable of absorbing light of a specific wavelength and transforming it into useful energy. Each factor is harmless by itself, but when combined, they can produce lethal cytotoxic agents that can selectively destroy cells. Thus, PDT may represent a promising alternative for reducing the bacterial load or even eradicating certain periodontal pathogens.[3] Photosensitizers penetrate gingival structures without causing any side-effects. Thus, by using PDT, it may be possible to eliminate bacteria such as P. gingivalis that have the ability to invade periodontal tissues.[4] PDT has become a potential treatment of infectious diseases with the development of laser medicine.[5]Thus the present study was aimed to evaluate and compare clinically and microbiologically, the efficacy of laser therapy and photodynamic therapy as an adjunct to scaling and root planing in the treatment of periodontal pockets.

2. Materials and methods

The subjects for the study were selected from amongst the outpatient department and each patient was given detailed verbal and written description of risks and benefits of the treatment with the consent to treatment agreement.

Subject selection[6,7]: Patients of both sexes, between the age group 25-60 years, who were diagnosed as cases of chronic periodontitis with atleast three sites, one in each quadrant with probing pocket depth of ≥ 5 mm and radiographic evidence of bone loss, exhibiting either positive or weekly positive BANA (Benzoyl-DL-arginine-B-napthylamide) test were included in the study. Patients having any systemic disease that could influence the outcome of periodontal therapy; undergone treatment for periodontitis or on anti- platelet or anti- coagulant therapy or systemic antibiotics during the last 6 months; smokers and alcoholics, pregnant or nursing women or any patient having any known allergy or hypersensitivity to any product used in the study were excluded from the study.

Study design

The present study was a single-blinded, split-mouth design clinical trial. Pre-operative protocol included detailed medical and dental history, microbiological assessment using Chairside BANA test kit (BANA-Zyme™ Reagent Strips, Oravital, Canada) was carried out. Periodontal parameters i.e. probing pocket depth (PPD), relative attachment level (RAL), plaque index,[8] gingival index[9]and sulcus bleeding index[10] were recorded. In each subject, at least three sites with probing pocket depth ≥ 5 mm in different quadrants were randomly allocated as Group A sites (scaling and root planing followed by laser therapy), Group B sites (scaling and root planing followed by photodynamic therapy) and Group C sites (scaling and root planing alone). Chairside BANA test and other clinical parameters were recorded at baseline and at two months.

Microbiological parameter: Chairside BANA test

The microbiological parameters were assessed using commercially available BANA test strips. Subgingival plaque sample was taken with the sterilized curette and placed on the reagent matrix affixed to the lower portion of the test strip. Upper part of the strip (salmon color) was slightly moistened with the distilled water using cotton swab. The strip was folded at the crease mark so that the lower and the upper reagent strips meet each other. Then it was kept in the pre-heated oven at 55 degree centigrade for 5 minutes and the color change was noted.

Interpretation of BANA Test-:

- Negative- No blue color observable.
- Weekly positive- Faint, pinpoint-size traces of blue color observable.
- Positive- Extensive obvious blue patches on pale red-brown background.

Treatment Protocol

The sites in selected patients were randomly assigned to one of the three treatment groups using chit method method. On first day, all subjects received routine oral hygiene instructions and one-stage full-

mouth scaling and root planing. On completion of scaling and root planing, Group A sites received laser therapy using 940 nm diode laser (Sirona, Germany) at 5.0 watts with 300 micrometer fibreoptic cable in a pulsed mode for 30 seconds. In Group B sites, periodontal pockets were filled with 1% Methylene Blue solution was applied deep into the pocket, kept for 3 minutes and then using 940 nm diode laser at 5.0 watts with 300 micrometer fibreoptic cable in a pulsed mode for 30 second, dye molecules were activated. The tip was initiated and introduced into the pocket with a smooth stroking action, starting coronally and working towards the bottom of the pocket. No more than 30 seconds were allocated to each tooth. On day seven, second dose of laser therapy and photodynamic therapy were repeated in respective periodontal pockets. Group C sites act as a control and were treated with scaling and root planing alone. Oral hygiene instructions were given and reinforced at regular intervals. Patients were then recalled after two months of the completion of therapy for the post-operative assessment of microbiological as well as clinical parameters.

3. Statistical analysis

Data compiled was put to statistical analysis.For intra-group comparisons, Wilcoxon signed rank test was used. For inter-group comparisons, Student's paired t-test was used.

Single investigator recorded all the readings. The investigator was trained and calibrated for all the methods. The intra-class correlation coefficient (ICC) between two measurements was calculated to assess the intra-observer variability. For all methods, the intra-observer variability was high (ICC = 0.87-0.94).

4. Results

All the subjects completed 2 months clinical trial, with no patient reporting any postoperative pain, discomfort or complications at any of the follow-up appointments. At the subject level, the overall periodontal condition was significantly improved after treatment. A total of 30 sites in 10 subjects were evaluated. At baseline, no significant differences were found between the three groups, while significant improvements in plaque index, gingival index and sulcus bleeding index were observed postoperatively at two months ($p < 0.05$) as shown in Table 1. In addition, a greater reduction in probing pocket depth and relative attachment level was reported in groups A and B ($p < 0.001$), shown in Table 2. On comparing the groups at 2 months postoperatively, there was no statistically significant difference between groups A and B ($p > 0.05$), whereas there was statistically significant difference in clinical parameters when group A and group B were compared to group C ($p < 0.05$) as shown in Tables 3 and 4. In case of microbiological parameter, thirty BANA-positive and weekly positive sites in ten subjects reduced significantly 2 months postoperatively in groups A and B i.e. 90% sites showed BANA-negative test, whereas in group C, only 50% sites showed such change as depicted in Table 5.

5. Discussion

The primary objective of initial periodontal therapy is the disturbance, disruption and control of the pathogenic plaque biofilms on the tooth surface.[11,12,13-15] Mechanical debridement can create significant changes in the microbiological environment of periodontal pockets by shifting the pathogenic biofilm to a beneficial one. This leads to a decrease in microbial loading and concentration of its products, such as lipopolysccharide, thereby resulting in a better control of host immune-inflammatory responses. Nonsurgical subgingival debridement significantly decreases the population of bacteria associated chronic periodontitis.[16,17] However, most of the pathogens including Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia and Treponema denticola are particularly resistant to the effects of subgingival debridement.[18] This has been linked with their ability to invade the pocket epithelium and underlying connective tissues.[16,19] Harboring pathogenic bacteria is associated with residual deep pockets, persistent bleeding and an increased risk of further disease progression.[20] Clinicians who recognize the impact of specific bacteria on periodontal conditions have incorporated antimicrobials both as systemic as well as local drug delivery as a part of periodontal therapy. However, frequent use of antimicrobials may lead to antimicrobial resistance and development of opportunistic infections, such as candidosis. Clinicians are therefore, in search of alternative adjunctive therapies that might provide similar benefits to antimicrobial therapy with fewer

side-effects. Dental lasers have shown to be potentially advantageous in non surgical and surgical periodontal treatments. They have been advocated in the removal of root surface deposits, soft and hard tissue ablation combined with haemostatic and bactericidal effects.[21,22,23] The antibacterial property of diode lasers against periopathogens has been recognized.[24,25] Photodynamic therapy combines the use of a photosensitizer with laser light to produce either free radicals or singlet oxygen molecules,[26] which have cytotoxic effect against periopathogens.[27] Photodynamic therapy has been claimed to have a broad spectrum of action, with efficacy against antibiotic-resistant strain without evidence of development of photoresistant strains, extensive reduction in the bacterial population and ability to target infected tissues.[28] Clinical studies combining photodynamic therapy with nonsurgical periodontal therapy have reported mixed outcomes.[29] Some studies showed that photodynamic therapy in combination with scaling and root planing led to a significant improvement in clinical parameters compared with scaling and root planing alone.[30,31,32,33] whilst others found that the adjunctive use of photodynamic therapy showed no significant benefits.[32,34,35]

Hydrolysis of trypsin substrate N-benzoyl-DL arginine-2-naphthylamide (BANA), from subgingival plaque samples, correlates best with the numbers and proportions of periodontopathogenic bacteria in plaque samples and may serve as an indicator of clinical disease.[36] The clinical management of patients with periodontal disease can be based on criteria given by both bacterial and clinical parameters that can be compared at different time intervals.[37]

In the present study, laser therapy as well as photodynamic therapy (laser + photosensitizer) has been used as an adjunct to scaling and root planing in the treatment of periodontal pockets. Initially, low-level laser therapy was provided by helium-neon gas lasers, but nowadays they have been replaced by gallium arsenide-based diode lasers.[38] The mechanism of laser therapy involves photoreceptors in the electron transport chain within the membrane of cell mitochondria. Absorption of light creates a short-term activation of respiratory chain components, promoting ATP production and activation of nucleic acid synthesis.[39] Laser therapy has an additional effect on fibroblasts by promoting proliferation and increasing cell numbers, secretion of growth factors and differentiation of fibroblasts into myofibroblasts.[40,41] This collectively results in improved wound contraction and accelerated wound healing.[35,38]

There have been recent reviews suggesting photodynamic therapy has limited effects on clinical parameters and subgingival bacteria loads.[29,34] As yet, no study has compared laser therapy and photodynamic therapy (laser + photosensitizer) as an adjunct to nonsurgical periodontal therapy using a chairside test kit. The present clinical trial shows that the adjunctive use of laser therapy and photodynamic therapy could significantly improve clinical as well as microbiological outcomes when compared to scaling and root planing alone. The overall improvement in clinical parameters viz. probing pocket depth, relative attachment level, gingival index, plaque index as well as sulcus bleeding index is far better when compared with the previous study.[30] The present study is well supported by the evidences provided by previous studies in which there has been a significant improvement in clinical, microbiological as well as biochemical parameters.[42,43,44]

Microbial biofilms in the oral cavity are involved in the progression of the periodontal disease progression. It was stated that the positive reaction of enzymatic BANA test manifests itself as an extensive obvious blue patches over the area in contact with plaque, indicating the presence of bacteria in a range of more than $5x10^5$ anaerobic CFU (colony forming units) at the site from where the sample was taken. The weak positive reaction of the enzymatic BANA test indicate the presence of pathogenic bacteria in a range of $1x10^5$ to $5x10^5$ anaerobic CFU and the negative BANA reaction manifests as a bacterial range of less than $1x10^5$ CFU at site from where sample was taken.[36]

In the present study, the results with the BANA test in groups A and B suggest a high reduction percentage in sites treated with laser therapy as well as photodynamic therapy, well supported by the evidences.[45,46] Most of the limitations reported in the previous studies viz. individual effects of laser therapy and photodynamic therapy has been covered and taken care off. There have been previous studies suggesting a single dose of laser as well as photodynamic therapy which has limited beneficial effect, so in the present study, two doses of such adjunctive measures has been advocated.[7]

Conclusion

Within the limitations of the study, both the laser and photodynamic therapy can be a beneficial adjunct to scaling and root planing in the treatment of periodontal pockets without the use of antibiotics thereby reducing the antibiotic consumption and its potential side effects. The ability of BANA test to detect anaerobic periodontal pathogens makes it an effective chair side method to diagnose and monitor the treatment efficacy in periodontal pockets and can help in easy detection of bacterial load both preoperatively and postoperatively and thus can be used as a good motivational tool for the patients for the maintenance of oral hygiene.

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TABLES

Table 1: Mean and mean difference in plaque index, gingival index and sulcus bleeding index in Group A, Group B and Group C
Note: Wilcoxon Signed Ranks test applied;

		Plaque Index			Gingival Index			Sulcus Bleeding Index		
	Assessment Interval	Mean ± SD	Mean difference from baseline	p value	Mean ± SD	Mean difference from baseline	p value	Mean ± SD	Mean difference from baseline	p value
Group A	Baseline	2.18 ± 0.24	1.85 ± 0.24	0.005*	2.35 ± 0.63	2.05 ± 0.55	0.005*	2.50 ± 0.53	2.00 ± 0.47	0.003*
	2 Months	0.33 ± 0.12			0.30 ± 0.28			0.50 ± 0.71		
Group B	Baseline	2.13 ± 0.27	1.78 ± 0.38	0.005*	2.23 ± 0.49	1.90 ± 0.39	0.005*	2.50 ± 0.53	1.90 ± 0.57	0.004*
	2 Months	0.35 ± 0.21			0.33 ± 0.21			0.60 ± 0.52		
Group C	Baseline	1.98 ± 0.55	0.68 ± 0.24	0.005*	2.03 ± 0.38	0.80 ± 0.16	0.002*	2.80 ± 0.79	0.60 ± 0.52	0.014*
	2 Months	1.30 ± 0.60			1.23 ± 0.42			2.20 ± 0.79		

Table 2: Mean and mean difference in probing pocket depth and relative attachment level in Group A, Group B and Group C

		Probing pocket depth			Relative attachment level		
	Assessment Interval	Mean ± SD	Mean difference from baseline	p value	Mean ± SD	Mean difference from baseline	p value
Group A	Baseline	6.10 ± 1.10	3.10 ± 0.74	<0.001**	9.70 ± 1.89	3.10 ± 0.74	<0.001**
	2 Months	3.00 ± 0.94			6.60 ± 1.90		
Group B	Baseline	5.70 ± 0.95	2.50 ± 0.53	<0.001**	9.40 ± 1.43	2.50 ± 0.53	<0.001**
	2 Months	3.20 ± 1.03			6.90 ± 1.66		
Group C	Baseline	6.40 ± 1.17	1.30 ± 0.82	0.001*	9.60 ± 1.27	1.30 ± 0.82	0.001*
	2 Months	5.10 ± 1.29			8.30 ± 1.34		

Note: Paired t-test applied;
* $p < 0.05$ = Significant p value; ** $p < 0.001$; highly significant p value

Table 3: Intergroup comparison of Change in plaque index, gingival index and sulcus bleeding index from baseline to 2 months between Group A, Group B and Group C

		Plaque Index		Gingival Index		Sulcus Bleeding Index	
Assessment Interval	Groups	Mean difference	p value	Mean difference	p value	Mean difference	p value
Baseline to 2 months	Group A	0.075 ± 0.57	0.719***	0.15 ± 0.43	0.327***	0.10 ± 0.74	0.655***
	Group B						
	Group A	1.18 ± 0.36	0.005*	1.25 ± 0.58	0.005*	1.40 ± 0.70	0.004*
	Group C						
	Group B	1.10 ± 0.46	0.005*	1.10 ± 0.34	0.005*	1.30 ± 0.68	0.006*
	Group C						

Note: Wilcoxon Signed Ranks test applied;
*$p < 0.05$ = Significant p value; *** $p > 0.05$= non significant p value

Table 4: Intergroup comparison of change in probing pocket depth and relative attachment level from baseline to 2 months between Group A, Group B and Group C

		Probing pocket depth		Relative attachment level	
Assessment Interval	**Groups**	**Mean difference**	**p value**	**Mean difference**	**p value**
Baseline to 2 months	**Group A**	0.60 ± 0.84	0.051***	0.60 ± 0.84	0.051***
	Group B				
	Group A	1.80 ± 0.80	<0.001**	1.80 ± 0.80	<0.001**
	Group C				
	Group B	1.20 ± 1.03	0.005*	1.20 ± 1.03	0.005*
	Group C				

Note: Paired t-test applied;
*p < 0.05 = Significant p value; ** p < 0.001 = highly significant p value; *** p > 0.05= non significant p value

Table 5: Change in number and percentage of positive and weekly positive tested sites to negative tested sites with BANA test at Baseline and at 2 months in Groups A, Group B and Group C

Groups	**Assessment Intervals**	**Number of BANA positive/ weekly positive sites**	**Number of BANA negative sites**	**Percentage change in BANA tested sites**
Group A	**Baseline**	10	0	90 %
	2 Months	0	9	
Group B	**Baseline**	10	0	90 %
	2 Months	0	9	
Group C	**Baseline**	10	0	50 %
	2 Months	0	5	