Antimicrobial Photodynamic Therapy using Indocyanine Green as a Photosensitizer in Treatment of Chronic Periodontitis: A Clinico-microbial Study

Abstract

Background: Photodynamic therapy (PDT) has gained much attention in recent years in the treatment of periodontitis. Number of photosensitizer have been developed and has been used in various clinical studies. However, the use of recently developed photosensitizer has been limited. Aim: The present study aims at comparing and evaluating the effects of photodynamic therapy using Indocyanine green in the treatment of chronic periodontitis. Materials and methods: In present randomized clinical trial, 30 subjects were equally divided into two groups i.e. test group (SRP + Photodynamic therapy) & control group (SRP). Clinical parameters evaluated at baseline and 3 month follow up were, Plaque index, Sulcus Bleeding Index, Probing Pocket Depth, Clinical Attachment Level, Gingival Recession. Microbiological analysis of plaque sample was also done to check for anaerobic mixed flora. Results: Significant reduction was seen in all the clinical parameters in the test group. Anaerobic culture of plaque samples of test group also revealed significant reduction of microorganisms in comparison with control group. Conclusion: Indocyanine Green can act as an alternative to other photosensitizers in photodynamic therapy as an adjunct to SRP in the treatment of chronic periodontitis.

Keywords: Antimicrobial photodynamic therapy, chronic periodontitis, indocyanine green, nonsurgical therapy

Introduction

Periodontal disease is a multifactorial disease, which causes inflammation of the supporting structures of the teeth caused by various periodontopathic bacteria leading to loss of teeth.[1] A primary objective of periodontal therapy is to remove soft and hard, supra- and sub-gingival deposits from the root surface to stop disease progression.[2] Plaque removal, which results in eradication of niches of periodontopathogens, is mainly performed using mechanical methods, such as nonsurgical therapy, which has resulted in significant clinical improvements as shown in previous studies.[3,4] In contrast, some studies have demonstrated that conventional mechanical therapy like scaling and root planing alone cannot completely remove all periodontal pathogens.[5] This may occur because of the presence of the anatomical complexity of the tooth roots, such as furcation areas and concavities, especially in deep periodontal pockets.[5,6] Thus, recolonization by those bacteria remaining in pockets or the tissues after mechanical debridement is a problem and this should be prevented by periodontal therapy.

To overcome these limitations of conventional mechanical therapy, various antimicrobial or antiseptic agents are used as an adjunctive to scaling and root planing (SRP) in the treatment of chronic periodontitis.[7,8] However, the efficacy of these adjutantive is limited due to the development of bacterial resistance.[9] As a result, there is a need to develop alternative antimicrobial approaches for periodontal treatment.

Over the years, a novel noninvasive photochemical approach for infection control, namely, photodynamic therapy (PDT) with the help of lasers has been receiving much attention in the treatment of oral diseases.[10,11] Use of lasers has been proposed as a new technical modality in the treatment of periodontal diseases.[12] Different types of lasers are used as an effective means
of decontamination of periodontal pockets over a period of years. Lasers are known to kill bacteria due to their high bactericidal properties, and they have demonstrated effective killing of oral pathogenic bacteria associated with periodontitis and peri-implantitis.[13] However, lasers may cause detrimental thermal effects on the surrounding periodontal tissues leading to potential and unexpected side effects.

Thus, recently, a new type of noninvasive phototherapy for bacterial elimination, called PDT, has been introduced, which uses low-level laser light and photosensitizer. The photosensitizer is a compound that is capable of absorbing light of a specific wavelength and transforming it into useful energy.[14] Each factor is harmless by itself; but when combined, they can produce lethal cytotoxic agents that can selectively destroy cells.[14]

Various photosensitizing agents such as methylene blue and chlorine are used at various wavelengths in periodontal therapy. Recent studies have shown improvement in clinical parameters and reduction in number of periodontopathogens such as Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis after the use of 810 nm laser activated indocyanine green (ICG).[15–19] ICG dye is a tricarbocyanine that belongs to the family of cyanine dyes and has been approved by the Food and Drug Administration for cardiovascular use and is used for measuring cardiac output, plasma volume, liver function, and ophthalmic angiography.[20,21] Some of its general characteristics are absorption in the infrared spectrum near 810 nm which was used in the present study, has low tissue toxicity, also rapid elimination and a very effective photosensitizer.[18]

Thus, the aim of the present study was to compare and evaluate the clinical and microbiological effect of PDT using ICG as a photosensitizer as an adjunctive to SRP in the treatment of chronic periodontitis.

**Materials and Methods**

A total of thirty participants (14 males, 16 females age range 30–55 years) diagnosed with chronic periodontitis were selected for the study from Outpatient Department of Periodontology. Ethical clearance was obtained from Institutional Ethical Committee and written informed consent was signed by the patients.

Inclusion criteria for the study were (i) chronic periodontitis patients with probing pocket depth (PPD) >5 mm and clinical attachment loss (CAL) >4 mm, (ii) systemically healthy controls, and (iii) patients not receiving any antibiotic therapy or had not received any periodontal therapy 12 months before the study. Exclusion criteria were patients with aggressive periodontists, pregnant and lactating females, smokers and tobacco chewers, and previous history of allergic reaction to the use of any kind of dye.

**Study design**

The present study was a randomized clinical trial. Clinical and microbial analysis was done at baseline and after 3 months. Clinical parameters which were evaluated at baseline and 1-month follow-up were plaque index (PI),[22] sulcus bleeding index (SBI),[23] PPD,[24] CAL,[24] and gingival recession (GR).[24] PPD was recorded using a University of North Carolina No. 15 periodontal probe (GDC®), and a custom-made acrylic stent was used to standardize the measurement of site-specific PPD [Figure 1]. CAL was calculated as the distance between the cementoenamel junction and base of the periodontal pocket. After examination, all the patients received full-mouth SRP using both hand instruments (Graze Curettes; Hu-Friedy) and ultrasonic device. After SRP, the 30 patients were randomly divided into two equal groups:

1. Control group: SRP only
2. Test group: PDT + SRP.

**Laser parameters**

The laser system used in the present study was diode laser (Biolase®, USA) with wavelength of 810 nm. The laser was applied in a continuous mode with a power of 0.8 W and irradiation time period of 60 s. Total energy produced was 5.4 J/cm² [Figure 2].

**Preparation of 5 mg/mL indocyanine green solution**

Under sterile conditions, ICG aqueous solutions are unstable and should be used within 24 h. Hence, whenever required, a fresh 5 mg/mL solution was prepared as follows: ICG (Aurogreen®, Aurolabs, Madurai, India) was dissolved in 5 mL of sterile water to prepare an initial 5 mg/mL ICG stock solution. The stock ICG solution was further diluted in saline solution at the ratio of 1:5 to achieve the final ICG concentration of 5 mg/mL [Figure 2].

**Microbial sampling**

Microbial sampling was done after SRP. The selected teeth were isolated with cotton rolls. A sterile paper point was inserted slowly with a sterile dental tweezer into the pocket until the tissue resistance was felt. The paper point was left for 30 seconds, and then, it was carefully removed without touching the adjacent unrelated tissues and placed into a special sterile test tube containing Robertson’s cooked meat broth medium. The medium was freshly prepared. To prevent the contamination of the media with the aerobic bacteria, the test tube was filled with 2 mL of paraffin oil; a cotton plug was placed. Samples were inoculated on blood agar plates [Figure 3]. Plates were incubated in an anaerobic GasPak™ at 37°C for 48 h [Figure 4].

**Treatment procedure**

After thorough instrumentation, the test group was exposed to PDT. Intrapocket delivery of 0.5–1 mL of ICG was done using a blunt needle. After a period of 60 s, pocket
was thoroughly rinsed using saline solution to remove excess photosensitizer from the pocket. Immediately after rinsing, the diode laser, with 810 nm wavelength and 0.8 W of power output, equipped with a probe tip, was placed at the depth of the pocket and moved circumferentially around the tooth for 1 min. However, in the control group, SRP was performed and no further treatment was provided [Figures 5 and 6].

**Statistical analysis**

A convenient sample size of thirty patients each having two contralateral sites was included under 5% alpha error and 80% of power of the test to detect the significant difference. All data collected were entered into Microsoft Excel (MS Office version 2010) and tabulated. Statistical analysis of the results was done using SPSS 20 (IBM Inc. Chicago, IL, USA). The Student’s t-test was used for
continuous variables after confirming normality of the data distribution. The method of Kolmogorov and Smirnov was used to confirm that the data were sampled from a Gaussian distribution. Statistical significance was defined as $P < 0.05$.

**Results**

All the patients enrolled in the study completed 3-month study. Healing took place without any complications. Patients in test group did not report any kind of side effects due to the use of photosensitizer like staining of the adjacent mucosa or tooth structure.

Demographic characteristics of the test and the control groups are shown in Table 1. At baseline, no significant differences in clinical parameters were noted between the test and the control patients [Table 2]. Mean ± standard deviation values for the clinical parameters in two groups at baseline and 3-month posttreatment are summarized in Table 2. There were no statistically significant differences between both groups with regard to changes in GR at any time point ($P > 0.05$). Regarding changes in PI, both the test and control group showed significant improvements after 3 months [Table 2]. In the test group, a statistically significant reduction ($P \leq 0.05$) in SBI, PPD, CAL was observed at 3-month posttreatment in comparison to control group.

Results of the bacterial culture and microscopic examination are shown in Figures 7-10. Marked reduction was noted in bacterial colonies after antimicrobial photodynamic therapy in the test group [Figures 7-10].

**Discussion**

Antimicrobial PDT was first utilized in medicine as a method for the treatment of cancer over a century ago.\cite{26} The principle of PDT involves the application of a photosensitizer to a target area, which is then activated by using light of specific wavelength, which produces reactive oxygen species causing cytotoxic effect on the target area. Different photosensitizer has specific absorption spectrum, for example, methylene blue-675 nm, toluidine blue O-675 nm, ICG-810 nm. Recently, many studies have been conducted where they have evaluated the efficacy of antimicrobial photodynamic therapy (aPDT) with different photosensitizing dyes most commonly methylene blue and toluidine blue O in periodontal treatment.\cite{27-29} However, there have been very few studies investigating the effects

![Figure 7: Microbial colonies after scaling and root planing](image1)

![Figure 8: Microbial colonies after photodynamic therapy + scaling and root planing](image2)

![Figure 9: The presence of Gram-negative rods after scaling and root planing](image3)

![Figure 10: Reduction in Gram-negative rods after photodynamic therapy + scaling and root planing](image4)
Sethi and Raut: Photodynamic therapy using indocyanine green in chronic periodontitis

Table 1: Demographic characteristics of the participants at baseline

|          | All    | Test   | Control |
|----------|--------|--------|---------|
| n        | 30     | 15     | 15      |
| Male/female | 14/16  | 7/8    | 7/8     |
| Mean age±SD | 47.8±5.10 | 50±2.83 | 44.90±5.32 |

SD=Standard deviation

Table 2: Comparison of mean±standard deviation of all clinical parameters at baseline and 3 months

| Clinical parameters | Groups | Baseline | 3 months | P     |
|---------------------|--------|----------|----------|-------|
| PI                  | Test group | 2.12±0.33 | 0.99±0.47 | <0.05 |
|                     | Control group | 2.08±0.27 | 1.28±0.45 | <0.05 |
| SBI                 | Test group | 3.28±0.48 | 0.57±0.53 | <0.05 |
|                     | Control group | 3.14±0.37 | 2.14±0.37 | >0.05 |
| PPD                 | Test group | 5.00±0.73 | 3.14±1.01 | <0.05 |
|                     | Control group | 5.30±1.20 | 4.60±0.60 | >0.05 |
| CAL                 | Test group | 6.11±0.64 | 4.70±1.08 | <0.05 |
|                     | Control group | 6.26±0.89 | 5.47±0.18 | >0.05 |
| GR                  | Test group | 1.14±0.55 | 1.55±0.56 | >0.05 |
|                     | Control group | 0.94±0.64 | 1.32±0.58 | >0.05 |
| P                   | >0.05 | <0.05 |

P>0.05 - nonsignificant, 0.05 - significant. PPD=Probing pocket depth, CAL=Clinical attachment level, GR=Gingival recession, SBI=Sulcus bleeding index, PI=Plaque index

of ICG as a photosensitizer as an adjunct to SRP in the treatment of chronic periodontitis.\textsuperscript{[17,18]}

The present randomized clinical trial evaluated the effects of ICG as a photosensitizer in chronic periodontitis patients clinically and microbiologically. Results of the present study suggest that there was a significant improvement in SBI, PPD, and CAL in test group as compared to the control group after 3-month posttreatment. Considering PI scores, both the groups showed significant improvement after three-month posttreatment. However, there was no significant difference between the two groups in relation to GR after a period of three months.

The present study showed significant improvements after three month in clinical parameters such as SBI, PPD, and CAL in the test group. Improvement in SBI scores in test group suggests that combination of PDT + SRP therapy results in better resolution of inflammation as compared to SRP alone. These results are in agreement with previous study done by Christodoulides et al. 2008\textsuperscript{[30]} where they found that PDT and SRP resulted in a significantly greater reduction in bleeding scores compared with SRP alone over a period of six months. Several other studies have also concluded that PDT and SRP has an added advantage over SRP alone in the reduction of bleeding on probing.\textsuperscript{[28,29]}

Significant reduction was also noted with respect to Probing Pocket Depth reduction in Test group as compared to Control group. The results of the present study regarding PPD reduction are in accordance with the previous studies.\textsuperscript{[28,29,31,32]} Alwaeli et al. 2015\textsuperscript{[32]} concluded that there was a significant reduction in pocket depth of 1.51 + 1.54 (mm) in test group as compared to control group where it was around 0.66 + 1.66. However, some studies contradict the changes in PPD reduction where they have concluded that PDT and SRP do not have any added advantage over SRP.\textsuperscript{[11,27,33]}

Considering the CAL scores, there was a significant gain in CAL in test group in the present study as compared to control group. In a randomized clinical trial by Andersen et al. 2007,\textsuperscript{[24]} significant gain in CAL was found in SRP and PDT group in comparison to SRP alone and PDT alone group. The resultant gain in CAL in the present study is consistent with findings of some other studies.\textsuperscript{[28,32,34,35]} However, there is still some controversy regarding the attachment gain after the application of PDT. This has been concluded in various studies where they have said that PDT does not have any effect on the attachment gain.\textsuperscript{[30,33]}

Considering PI scores, there was no additional benefit found from a PDT as an adjunctive treatment for patients with chronic periodontitis. There was a significant improvement in PI scores in both the groups after three-month period. However, it needs to be kept in mind that changes in PI scores are dependent on patients’ compliance and the fact that the PI scores were improved significantly in both groups in the present study suggests that patients had properly maintained the oral hygiene.

In the present study, microbiological analysis for the anaerobic bacteria present in the periodontal pocket was also done. Visual examination of bacterial culture and microscopic examination by Gram’s staining method were also done to evaluate the effects of PDT and SRP. Marked reduction was noted in the bacterial colonies after PDT and SRP, and their microscopic examination also revealed reduction in anaerobic bacterial rods present in the periodontal pocket. However, statistical analysis of the microbiological results was not done.

To the best of our knowledge, until now, only two studies have been conducted evaluating the effect of ICG as a photosensitizer in the treatment of chronic periodontitis. The results of the present study are consistent with previous studies using ICG as a photosensitizer.\textsuperscript{[17,18]} Regarding the mechanism of action of ICG, the effects are mainly thought to be photothermal and photochemical. ICG is known to enhance the photothermal effects of high penetration 810 nm diode lasers; thus, potentiating the benefits of lasers. Therefore, 810 nm diode laser along with ICG can act as a potential photosensitizer in the treatment of chronic periodontitis.

However, it should be noted that the results of the present study are difficult to compare with previous studies as there is a lack of standardization regarding the use of PDT in the treatment of chronic periodontitis. This is because of
different types of photosensitizers used, different types of wavelengths of lasers with different power output and irradiation time. Thus, there is a need for further studies with standardized treatment protocol using lasers.

The present study has some limitations that need to be taken into consideration before interpreting the results. These are limited sample size and lack of evidence on the effect of PDT against specific bacterial strains. Furthermore, there is limited literature regarding the effective concentration of Indocyanine green to be used as photosensitizers in the treatment of periodontitis.

Thus, further randomized controlled clinical trial needs to be conducted evaluating the effect of ICG as a photosensitizer with a larger sample size and longer study period. In addition, its effects can be evaluated in conditions such as aggressive periodontitis and peri-implantitis.

Conclusion
It can be concluded that ICG combined with an 810 nm diode laser may be used as an adjunct to SRP in the treatment of chronic periodontitis.

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Conflicts of interest
There are no conflicts of interest.

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