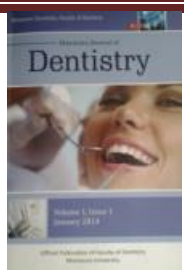

The use of Diode laser with photosensitizer (Indocyanine Green) in Treatment of chronic Periodontitis patients (clinical & microbiological study)

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Abstract:

Objectives: The aim of the present study is to evaluate the clinical effect of Indocyanine Green photosensitizer activated by Diode laser as an adjunctive treatment to non-surgical periodontal therapy and it's antimicrobial effect on porphyromonas gingivalis and prevotella intermedia

Patients and methods: Thirty patients of both sexes over the age of 30 who were diagnosed with chronic periodontitis were selected. Medical and dental history were taken from the patients. Evaluation of clinical parameters was performed 1. Plaque index. 2. Clinical attachment level. 3 pocket probing depth 4 Bleeding index. The microbiological evaluation was performed by obtaining samples of gingival crevicular fluid. All patients were treated with full scaling and root planing. Then the patients were divided into the two groups: Group 1): consisted of fifteen patients. Application of Photodynamic therapy using diode laser combined with indocyanine photosensitizer. Group 2): consisted of fifteen patients, treated by scaling and root planning. Clinical and microbiological evaluation was performed at the baseline and after six weeks

Results: All clinical parameters (PI, SBI, PPD and CAL), total bacterial count in addition to P.gingivalis & *P.intermedia* counts were revealed statistically significant difference after six weeks of treatment among both groups. However on comparing post-treatment values of *P.gingivalis & P.intermedia* counts in between groups demonstrated statistically significant difference between groups 1&2.

Conclusions: The Indocyanine Green Photodynamic therapy offers a promising therapeutic approach in periodontal treatment as adjunct to SRP.

Keywords: *antimicrobial photodynamic therapy, indocyanine green, periodontitis, scaling and root planing*

Introduction

Periodontitis is a multifactorial disease that is associated with loss of the supporting tissues (i.e., periodontal ligament and alveolar bone) around the tooth (1). Periodontitis is caused by a pathogenic microbiota in the subgingival biofilm, including *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, prevotella* intermedia and Treponema denticola that trigger innate, inflammatory, and adaptive immune responses. These processes result in the destruction of the tissues surrounding and supporting the teeth, and finally, tooth loss (2).Removal of the biofilm and elimination of periodontal pathogens from the periodontal pocket is the main purpose of treatment for this disease (3).

Photodynamic therapy (PDT) is a clinical modality of photochemotherapy based on the accumulation of a photosensitizer in target cells and subsequent irradiation of the tissue with light of adequate wavelength promoting reactive oxygen species (ROS) formation and cell death (4). Recently a new photosensitizer called Indocyanine green (ICG), is a tri-carbocyanine that belongs to the large family of cyanine dyes (5).The ICG molecule exhibits a molecular structure with amphiphilic properties that has both hydrophilic and lipophilic properties. Through photon-induced electron transfer, ICG is able to produce powerful photosensitized cellular damage (6, 7).

ICG has proven effectiveness as a light-activated antibacterial agent, for adjunctive use in wound healing or treating chronic infections of mucous membranes and skin. When photo-excited, ICG can induce the production of singlet oxygen with strongly cytotoxic activity (8).

ICG in therapeutic concentrations has almost no host toxicity and is approved by the USA FDA for medical applications (9). It has been investigated for use in bacterial infections (10, 11) and within the treatment of antibiotic resistant bacterial pathogens, ICG has been investigated against selected bacterial species (*S. aureus* and *P. aerugi-nosa*) *in vitro*, providing statistically-significant reduction in bacteria of 95–99%, depending on fluence values (12)

ICG diode laser activated could be a promising adjunctive therapy in the treatment of periodontitis need more clinical trials

Patients, materials and methods:

A total of thirty patients above 30 years old were diagnosed with chronic periodontitis and selected from the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University. All patients signed an informed consent and they were aware of the purpose of the study

- **The patients were divided by the following groups :Group (1) Photodynamic Therapy(PDT)+SRP:** Comprised of fifteen patients were treated once or twice by full scaling and root planning Application of diode laser with indocyanaine green after one week.

Group (2) SRP: Comprised of fifteen patients were treated with scaling and root planning only

- **Assessment: Clinical Assessment:** Clinical parameters were taken at the baseline and after 6 weeks including: Plaque index (**PI**), Clinical attachment Level (**CAL**), Periodontal Probing Depth (**PPD**), Bleeding Index (**BI**)**. Microbiological assessment:** were taken at the baseline and after 6 weeks**.** ***P.gingivalis and P. intermedia*** **Identification:** Morphological and Microscopic Identification. Biochemical Reactions: include Glucose Fermentation., Motility Test, Catalase Test, Indole Test, Urease Test**.** ***P,gingivalis and prevotella intermedia*** **colony count (CFU).**
- **Treatment procedure: Group (1) Photodynamic therapy (PDT)+SRP:** The laser system used in the present study was diode laser with wavelength of 810 nm. The laser was applied in a continuous mode with a power of 0.5 W and irradiation time period of 30 s. Total energy produced was 5.4 J/cm 2**.** Syringe with a blunt cannula which was then used to fill the periodontal pocket with the dye. Periodontal pockets were rinsed with dye starting from the bottom of pocket to achieve complete filling of the pocket and coating of the root surface (18). After 3 min, the patient was asked to rinse with water to remove excess photosensitizer. Immediately after rinsing, the diode laser, with 810 nm wavelength and 0.5 W of power output, equipped with a probe tip, placed at the depth of the pocket and moved circumferentially around the tooth for 30 seconds, according to the manufacturer's instructions. **Group (2) SRP:**The fifteen patients were treated with scaling and root planning

Analytical statistics:

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 24. Numerical data were summarized using means and standard deviations or medians and ranges. Data were explored for normality using Shapiro-Wilk test. Categorical data were summarized as number and percentages. Comparisons between the 2 groups with respect to normally distributed numeric variables were done using the student t test and Paired t test. None normally distributed numeric variables were compared by Mann Whitney test and Wilcoxon signed rank test. Comparison overtime was done by paired t test and its non-parametric analogue Wilcoxon signed rank test as appropriate**.** Spearman correlation coefficient was used to correlate between non parametric continuous variables (clinical indices and microbiological results). All p-values are two-sided. P-values ≤ 0.05 were considered significant

Results:

A total of thirty patients above 30 years old were diagnosed with chronic periodontitis and selected from the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, fifteen in group I (PDT & SRP) and fifteen in group II (SRP). Both groups were followed up for six weeks to assess clinical indices and microbiological results.

Clinical Parameters:

Plaque index: At the baseline*:* for Group I; the mean plaque index was 2.5 ±0.6 while for group II was 2.5 ±0.5. After 6 Weeks it was 0.6 ±0.5 and 0.5 ±0.7respectively. **Periodontal Probing depth:** At the baseline: for Group I; the mean Probing depth was 5.7 ± 0.9, for group II was 5.6. ± 0.8. After 6 weeks it was 3.9 ±.0.8 and 4.7 ±0.8 respectively. **Bleeding Index:** At the baseline: for Group I; the mean bleeding index was 2.7±0.5, for group II was 2.6±0.5. After 6 weeks it was 0.5±0.5 and 0.5±0.6 respectively. **Clinical Attachment Level (CAL):** At the baseline: for Group I; the mean CAL was 5.4 ± 1.1, for group II was 5.9 ± 1.2. After 6 weeks it was 3.7 ± 0.8 and 4.8 ± 1 respectively.

Microbiological results

***P.gingivalis*:** At the baseline: for Group I; the mean P. gingivalis colonies count was 49.13 ± 10.75, for group II was 49.53 ± 9.11. After 6 weeks it was 11.6 ±2.79 and 22.87 ± 1.85 respectively. **P.intermedia:** At the baseline: for Group I; the mean P. intermedia colonies count was 45.2 ± 3.08 , for group II was 45.73 ± 2.91 **.**After 6 weeks it was 5.47 ± 1.25 and 13.87 ± 1.55 respectively.

Discussion

The present study was a single‐blind randomized clinical trial, which evaluated the effects of ICG‐mediated photodynamic therapy in chronic periodontitis patients clinically and microbiologically.

In the present study, the patients did not report any adverse effect posttreatment. It was also noted that ICG did not stain the teeth or restorations with the exception of plaque, which stained green, presumably due to the bacterial content.

PDT was carried out 1 week after the completion of SRP. The rationale behind this was that a bleeding sulcus would have a reductive effect on the dye penetration into the pocket. The dye would be rinsed from the sulcus or diluted to invalid levels by the bleeding, which would end up neutralizing its effect completely. This might affect the final treatment outcome; hence, a time shift was recommended in this treatment.

In the present study considering Plaque **Index** (**PI)** scores, there was a significant improvement in PI scores in both groups after six weeks period in comparison to the baseline.

On comparing the **PI** scores of these results in the two groups, there was no statistically difference found from PI scores in the test group compared to control group after six weeks.

The present results were in accordance with the results obtained in studies conducted on adjunctive PDT by Christodoulides et al, and Theodoro et al., in which the intergroup comparison of PI scores yielded no statistically significant difference (1, 2).

In the present study considering **Sulcus Bleeding Index (SBI)** scores we found that there was no significant difference in both groups when comparing to each other after six weeks.

These were in agreement with K Joshi et.al, which found no statistically difference between groups after 6 weeks (1, 2)

These results of the present study were in consistent with previous study done by Monzavi *et al*., 2007,where they found that PDT and SRP resulted in a significantly greater reduction in bleeding scores compared with SRP over a period of 6 weeks(3).

Considering the **periodontal probing depth (PPD)** and the **clinical attachment** level **(CAL)** scores, we found that there was a significant difference in PPD reduction and CAL gain in the test group compared to control group after sex weeks.

The present results were in agreement with Andersen *et al*.and Alwaeli *et al*., 2015 who reported an improvement in the Periodontal probing depth and clinical attachment level in the group treated with SRP alone after 6 weeks [(5)].

The present results were in consistent with results reported by Petelin et al. who stated that there was no significant difference in PPD reduction and CAL gain (9).

Concerning **microbiological evaluation**, on comparing the total bacterial count, *P.gingivalis* and *P.intermedia* count between the test and control group , there was a significant reduction in bacterial colonies count (in CFU) in the test group compared to control group after six weeks

This significant reduction in total bacterial count may be attributed to the high bactericidal effect of indocyanine green photodynamic therapy activated by an 810 nm diode laser in absence of oxygen, making its effect on anaerobic bacteria was great. Moreover ICG has a wide optical absorption band from 600 to over 800 nm with optimal peak at 805–810 nm near-infrared wavelength, this wavelength of 805–810 nm has more capacity to penetrate biological tissue than rest of the spectrum. Penetration depth in biological tissue for visible-red wavelengths (650 nm) is 3–3.5 mm, whereas for near-infrared light (800–1100 nm), it reaches up to 6 mm (13)

The mechanism of uptake of ICG by periodontal bacteria appears to be unclear. However, it has been demonstrated that this uptake was more specific to periodontal bacteria compared to gingival cells which take up 10 times lesser amounts of ICG (14, 15).

While scaling and root planing have many limitations, including the inability to adequately instrument deep periodontal pockets and furcations as well as removing microorganisms within the tissue lining the periodontal pockets leading to recolonization of bacteria in the periodontal pocket, making scaling and root planing insufficient for pocket disinfection.

These finding were in agreement with the previous studies done by Srikanth *et al*, where they found that there was a significant reduction in the amount of anaerobic pathogens in PDT group. Although this was quantitative estimation, still an important factor as a reduction in total anaerobic bacterial load is a major determinant of periodontal health (16).

Although few studies conducted, evaluating the effect of PDT on specific pathogen has demonstrated contradictory results (12, 18). One possible reason for this may be the inability of other photosensitizers (methylene blue and toluidine blue) to get activated in anaerobic environment subgingivally as compared to ICG which can get activated without oxygen.

However, it is important to emphasize that the clinical conditions such as time of performance and tissue photosensitizer concentration, pH change, exudate presence, and gingival fluid in the sub-gingival environment can influence the effectiveness of therapy (45). Thus, the comparison between different studies is challenged by various laser parameters, photosensitizers' concentrations, and changes in periodontal conditions and periodontal treatments.

Conclusion

Based on the results of the study it can be concluded that:

- Indocyanine green photodynamic therapy as an adjunct to SRP has resulted in significant additional improvement in the clinical conditions of moderate chronic periodontitis patients when compared with SRP alone. Iindocyanine green dye is absorbed in the infrared spectrum which allows better tissue penetration and has been found to be effective against periodontal pathogens even at low concentration. Indocyanine green photodynamic therapy has a bactericidal effects on anaerobic subgingival pathogens

Table (1): Comparison of Plaque index at the baseline and after 6 weeks between studied groups.

Plaque index	Group I n=15	Group II n=15	p value 2
	Mean±SD	Mean±SD	
Baseline	2.5±0.6	2.5±0.5	0.870
6 Weeks	0.6±0.5	0.5±0.7	0.595
p value 2	<0.001	0.001	

SD: standard deviation , p≤0.05 is significant , p value 1 for comparing baseline with 6 weeks (overtime) in each single group done by Wilcoxon signed rank test, p value 2 : for comparing between groups at different time points done by Mann Whitney U test

Table (2): Comparison of bleeding index at the baseline and after 6 weeks between studied groups.

Bleeding Index Median(Range)	Group I n=15	Group II n=15	p value 2
	Mean ± SD	Mean ± SD	
Baseline	2.7±0.5	2.6±0.5	0.775
6 Weeks	0.5±0.5	0.5±0.6	0.902
P value 1	<0.001	0.001	

SD: standard deviation , p≤0.05 is significant , p value 1 for comparing baseline with 6 weeks (overtime) in each single group done by Wilcoxon signed rank test, p value 2 : for comparing between groups at different time points done by Mann Whitney U test

Table (3): Comparison of periodontal probing depth at the baseline and after 6 weeks between studied groups.

Periodontal Probing Depth (mm)	Group I n=15	Group II n=15	P-value 2
	Mean ± SD	Mean ± SD	
Baseline	5.7±0.9	5.6±0.8	0.836
6 Weeks	3.9±0.8	4.7±0.8	0.010
P value 1	<0.001	<0.001	

SD: standard deviation, p≤ 0.05 is significant, p value 1 for comparing baseline with 6 weeks (overtime) in each single group ,Used test: Paired t test

P value2 for comparing between groups at different time points done by independent t test

Table (4): Comparison of Clinical Attachment Level at the baseline and after 6 weeks between studied groups.

	Clinical Attachment Level (mm) Mean±SD		p value 2
	Group I n=15	Group II n=15	
Baseline	5.4±1.1	5.9±1.2	0.282
6 Weeks	3.7±0.8	4.8±1.0	0.003
p value 1	<0.001	<0.001	

SD: standard deviation, p≤ 0.05 is significant, p value 1 for comparing baseline with 6 weeks (overtime) in each single group. Used test : paired t test

P value 2: for comparing between groups at different time points .Used test :independent t test

Table (5): Comparison of *P. gingivalis* colonies count (CFU) in the studied groups at the baseline and after 6 weeks

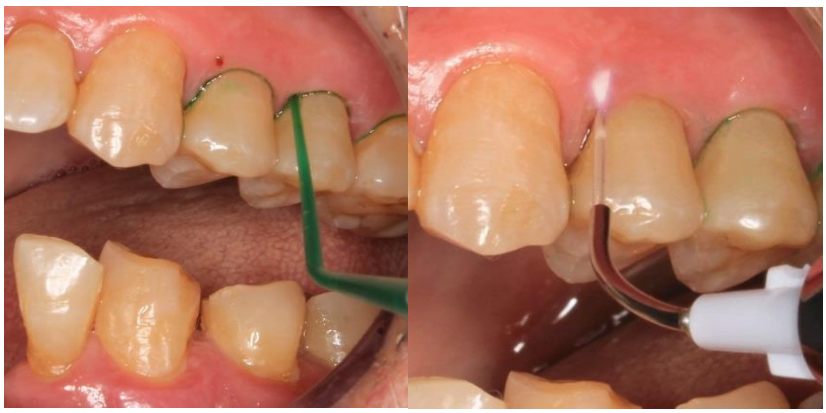
	P.Gingivalis Mean ± SD		p value 2
	Group I n=15	Group II n=15	
Baseline	49.13±10.75	49.53±9.11	0.913
6 Weeks	11.60±2.79	22.87±1.85	<0.001*
p value 1	<0.001*	<0.001*	

SD: Standard deviation , p<0.05 is significant, p value 1 for comparing baseline with 6 weeks (overtime) in each single group done by paired t test .P value 2: for comparing between groups at different time points used test independent t test.*statistically significant .CFU: colony forming unit

Table (6): Comparison of *P. intermedia* colonies count (CFU) in the studied groups at the baseline and after 6 weeks

	P.intermedia Mean ± SD		p value 2
	Group I n=15	Group II n=15	
Baseline	45.20±3.08	45.73±2.91	0.630
6 Weeks	5.47±1.25	13.87±1.55	<0.001*
p value 1	<0.001*	<0.001*	

Standard deviation , p<0.05 is significant, p value 1 for comparing baseline with 6 weeks (overtime) in each single group done by paired t test .P value 2: for comparing between groups at different time points used test independent t test.*statistically significant. CFU: colony forming unit



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