Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 363-371
Received 15 April 2021; Accepted 05 May 2021.

Indocyanine Green-Mediated Photodynamic Therapy as Adjuvant to Non-Surgical Periodontal Treatment in Chronic Periodontitis Patients: A Clinical and Microbiological Study

Running title: Indocyanine green-mediated photodynamic therapy as adjunctive treatment of chronic periodontitis.

[1]Dr. Padmaja Vangipuram, [2]Dr. PL Ravishankar, [3]Dr. AV Saravanan, [4]Dr. Jasmine Vaidya, [5]Dr. G Visithiriyan, [6]Dr. Preethikaa Gurupasath

1. M.D.S., Postgraduate student, Department of Periodontics, SRM Kattankulathur Dental College &Hospital, Chennai, India
2. M.D.S., Professor & HOD, Department of Periodontics, SRM Kattankulathur Dental College & Hospital, Chennai, India
3. M. D.S., Reader, Department ofPeriodontics, SRM Kattankulathur Dental College &Hospital, Chennai, India
4. M.D.S., Postgraduate student, Department of Periodontics, SRM Kattankulathur Dental College &Hospital, Chennai, India
5. M.D.S., Postgraduate student, Department of Periodontics, SRM Kattankulathur Dental College &Hospital, Chennai, India
6. M.D.S., Postgraduate student, Department of Periodontics, SRM Kattankulathur Dental College &Hospital, Chennai, India

Department(s) and institution(s): Department of Periodontics, SRM Kattankulathur Dental College &Hospital, SRM Institute of Science and Technology, Chennai, India

Corresponding Author:
Dr. Padmaja Vangipuram

Address: Postgraduate student, Department of Periodontics, SRM Kattankulathur Dental College & Hospital, SRM Institute of Science & Technology, SRM Nagar, Kattankulathur, Kanchipuram, Chennai - 603203, TN, India.

Indocyanine Green-Mediated Photodynamic Therapy As An Adjuvant To Non-Surgical Periodontal Treatment In Chronic Periodontitis Patients: A Clinical And Microbiological Study

Abstract

Aim: To evaluate the efficacy of indocyanine green-mediated photodynamic therapy as an adjunctive therapy to scaling and root planing by evaluating clinical and microbiological parameters in the treatment of chronic periodontitis.

Materials and methods: Thirty-foursystemically healthy patients were screened. In this split-mouth study, contralateral sites were allotted to Group 1 (Scaling and root planing) and Group 2 (Scaling and root planing with photodynamic therapy). Indocyanine green dye (Aurogreen™) and diode laser at 810nm were used for antibacterial photodynamic therapy. Subgingival plaque samples were obtained from the patients at baseline and three months after treatment. Clinical parameters (PPD, CAL, PI, GI and mSBI) were recorded both at baseline and after three months. The plaque samples were then subjected to microbial culture on blood agar and colony forming units were counted.

Statistical analysis: Results were statistically analysed using Mann Whitney test and Wilcoxon Signed Rank Test.

Results: All clinical and microbiological parameters showed statistically significant reduction following Phase I therapy after a period of three months in both groups. There was also a statistically significant improvement in all parameters in Group 2 (SRP+PDT) when compared to Group 1 (SRP) at the end of the treatment period.

Conclusion: Indocyanine green-mediated photodynamic therapy has a significant role as an adjuvant in the non-surgical management of chronic periodontitis and is shown to be better than SRP alone.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 363-371
Received 15 April 2021; Accepted 05 May 2021.

Keywords: antibacterial, chronic periodontitis, indocyanine green, non-surgical periodontal therapy, photodynamic therapy

Introduction

Periodontitis is a multifactorial disease that is associated with destruction of the supporting tissues around the tooth.[1] One major objective of periodontal therapy is to remove soft and hard, supra- and subgingival deposits from the root surface in order to stop disease progression.[2] Conventional periodontal therapy includes both surgical and non-surgical approaches that involve instrumentation of the inflamed dento-gingival complex.Of the currently available instrumentation techniques, none are effective in completely eliminating subgingival bacteria and calculus, although non-surgical therapy has been shown to result in significant clinical improvements.Photodynamic therapy (PDT) entails the inactivation of microorganisms or molecules by a combination of visible light, usually in the form of a laser, and a photosensitizer. The photosensitizer is a molecule that iscapable of absorbing light of a specific wavelength and producing lethal cytotoxic agents that can then destroy cells.Photodynamic therapies require two components. This includes a visible light source usually of a specific wavelength and a dye or photosensitizer that binds to the target cell and is activated by a light source.[3]The concept of photodynamic therapy can cause lethal photosensitization of Porphyromonas gingivalis and other periodontopathogenic bacteria and may lead to decreased bone loss in periodontitis patients.Recently, a novel photosensitiser called indocyanine green (ICG), a tricarbocyanine that belongs to the family of cyanine dyes has been developed. It has numerous applications in medicine such as to determine cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography. Recently conductedin vitro studies have reported its efficacy in eliminating known periodontal pathogens.

Thus, the purpose of the current study was to investigate the efficacy of photodynamic therapy using Indocyanine green (AurogreenTM) as an adjunct to non-surgical periodontal therapy on periodontal pathogens in the management of chronic periodontitis.

Materials and methods

The present study was a clinical and microbiological study. Thirty-four chronic periodontitis subjects were chosen for the study according to the classification of periodontal diseases and conditions given by American Academy of Periodontology (AAP) in 1999.[4]

The study protocol was discussed and accepted by the Institutional Ethical Committee and Review Board. Prior to involvement, the purpose and procedures were fully explained to the subjects, and all participants gave written and verbal informed consent. The subjects participated for up to three months with two visits in total; baseline and 3 months post-therapy.

The inclusion criteria were patients who were diagnosed with chronic periodontitis, and were in the age group of 35-55 years (according to Armitage's criteria given in 1999), those with periodontal pockets on contralateral sites with probing depth of >5 mm and CAL >4mm and systemically healthy patients. Exclusion criteria were patients with aggressive periodontitis, medically compromised patients, pregnant and lactating women, patients who have received any periodontal therapy (non-surgical or surgical) six months prior to the initial examination, those who used antibiotic drugs within six months of baseline examination, teeth with hopeless prognosis, smokers and alcoholics and patients with known allergy to Indocyanine green.

Sixty-eight sites (Thirty-four subjects) were randomized to either Group 1, which received only scaling and root planing (SRP alone) and Group 2 which received photodynamic therapy as an adjunct to scaling and root planing (SRP+PDT).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 363-371
Received 15 April 2021; Accepted 05 May 2021.

The screening/baseline data collection was done at the first visit in which basic medical and dental information was obtained. The following parameters were recorded:Probing Depth (PD)[5], Clinical attachment loss (CAL)[6], Plaque index (PI)[7], Gingival index (GI)[7] and Modified Sulcular Bleeding index (mSBI)[8].

Non-surgical periodontal therapy

Upon completing the exam, it was followed by the scaling and root planing of control and test sites by hand (a variety of Gracey curettes, Hu-Friedy) and ultrasonic instrumentation under local anesthesia. All debridement was performed by the same clinician with no time restriction.

Microbiological assessment

Plaque samples were collected from the periodontal pocket on contralateral sides at baseline and three months post-op. Supragingival calculus and plaque were carefully removed, following whichthe selected site was dried and isolated using cotton rolls. Then, sampling was done using a sterile paper point which was inserted and left in place for 30 seconds. It was then transferred to 1ml of Robertson's cooked meat broth medium. This was subsequently incubated for two hours.

The samples were then serially diluted and 100 microlitre of diluted specimen was streaked onto culture medium [blood agar with Hemin (5mg/ml), Kanamycin and Vitamin K (10mg/ml)] and cultured using anaerobic jar at 35-370 C for three days.

All samples were inspected for total anaerobic bacterial colony count using a digital colony counter. The presumptive identification of various bacteria was made on the basis of colony morphology and aero-tolerance (Figures 4 and 5).

Photodynamic therapy

Laser settings

Soft tissue diode laser (Zolar, Canada) was used in this study. The wavelength was 810 nm. Irradiation was done in a continuous setting. Power of 0.8 W and time of 60s were used. The energy generated during the procedure was 5.4 J/cm2.

Preparation of 1mg/ml dye solution

Aurogreen® (Figure 1) manufactured by Aurolabs, Madurai, India is a commercially available Indocyanine green dye. The solutions are unstable in sterile conditions. It was prepared as and when needed according to the method as follows: 25 mg ICG was dissolved in a 5ml of sterile water. This resulted inICG stock solution with an initial concentration of 5 mg/ml which was diluted in sterile saline so as to achieve a final concentration of 1 mg/ml.

In Group 2 (test group) the prepared solutionwas applied with a blunt needle to the selected sites. Application was begun in an apico-coronal direction so as to avoid air bubbles being entrapped (Figure 2).After waiting for three minutes, the area was rinsed with sterile saline. Then, diode laser with a probe tip was placed into the pocket and moved around the tooth for one minute (Figure 3).

Follow-up visit

The second visit was the three-month re-evaluation exam. The same complete exam as in baseline visit was performed by the same examiner. Any adverse events and concomitant medications were recorded. Subjects maintained their usual oral hygiene procedures throughout the course of the study. Microbial sampling was done as in baseline appointment.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 363-371
Received 15 April 2021; Accepted 05 May 2021.

Statistical analysis

All the clinical and microbiological data were collected and transferred into a Microsoft Excel spreadsheet. The data were analyzed with SPSS 20.0 version software programme. Mann Whitney test was used for intergroup comparison between Group 1 and Group 2 of all the parameters at the same time period. Wilcoxon signed rank test was used to compare the parameters between the baseline and three months post-op period within both groups. In the above statistical analysis, probability value below 0.05 was considered to be statistically significant.

Results

In the present study, a total of thirty-four patients diagnosed with chronic periodontitis have participated. Clinical parameters were measured and plaque samples were collected at baseline and three months post op. The samples were divided into four groups, namely, Group 1 T0, Group 1 T3, Group 2 T0 and Group 2 T3, where T0 stands for baseline and T3 for three months post-op. The comparisons for the variables were done to assess the pre-op and post-op effects of scaling and root planing and the added benefit of photodynamic therapy.

The results were considered statistically significant if $p \leq 0.05$ and non-significant if $p \geq 0.05$.

Clinical parameters

The clinical parameters (PPD, CAL, PI, GI and mSBI) of the patients both at baseline and three months post-operative period are summarised in Tables 1 and 2. They are also graphically represented in Figures 6 and 7.

Microbiological parameters

The Colony Forming Units of black pigmented bacteroids on blood agar cultures were counted. The data are seen in Tables 1 and 2 and Figure 8.

Table 1: Comparison of mean values of parameters between two groups at same time intervals by Mann Whitney test (Intergroup)

Parameter	Time interval	Group 1 Mean±SD	Group 2 Mean±SD	Mann Whitney test	"p" value
PPD (mm)	T0	5.54±0.31	5.55±0.33	109.000	0.884
	T3	4.05±0.233	2.69±0.31	0.000	0.0001
CAL (mm)	T0	4.35±0.22	4.36±0.31	110.000	0.916
	T3	3.49±0.30	2.25±0.15	0.000	0.0001
PI	T0	1.81±0.38	1.77±0.38	101.000	0.630
	T3	1.49±0.33	0.68±0.22	7.500	0.0001
GI	T0	2.07±0.25	1.99±0.27	90.000	0.345
	T3	1.70±0.24	0.69±0.20	0.000	0.0001
mSBI	T0	2.23±0.26	2.33±0.28	87.500	0.294
	T3	1.95±0.28	0.80±0.19	0.000	0.0001

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 363-371
Received 15 April 2021; Accepted 05 May 2021.

CFU (log)	T0	9.05±0.31	9.08±0.27	108.000	0.849
	T3	8.45±0.23	6.03±0.82	0.000	0.0001

Table 2: Comparison of mean values of the parameters at different time intervals by Wilcoxon signed rank test (Intragroup)

Parameter	Group	T0 Mean±SD	T3 Mean±SD	Wilcoxon signed rank test	"p" value
PPD (mm)	G1	5.54±0.31	4.05±0.23	3.420	0.001
	G2	5.55±0.33	2.69±0.31	3.413	0.001
CAL (mm)	G1	4.35±0.22	3.49±0.30	3.415	0.001
	G2	4.36±0.31	2.25±0.15	3.427	0.001
PI	G1	1.81±0.38	1.49±0.33	3.342	0.001
	G2	1.77±0.38	0.68±0.22	3.417	0.001
GI	G1	2.07±0.25	1.70±0.24	3.432	0.001
	G2	1.99±0.27	0.69±0.20	3.420	0.001
mSBI	G1	2.23±0.26	1.95±0.28	3.460	0.001
	G2	2.33±0.28	0.80±0.19	3.417	0.001
CFU (log)	G1	9.05±0.31	8.45±0.23	3.214	0.001
	G2	9.08±0.27	6.03±0.82	3.409	0.001

Pocket probing depth

The mean values of PPD were higher in Group 2 at baseline but post therapeutic values were higher in Group 1. Both the groups showed a statistically significant reduction of mean probing depth values from baseline to three months visit with p = 0.001. The difference between the groups was statistically significant with much lower values in Group 2 at post op visit with p = 0.0001.

Clinical attachment loss

The mean CAL values were lower post therapy in Group 2 as compared to Group 1. Comparison showed a statistically significant difference with a p value = 0.0001 in favour of Group 2. Clinical Attachment Loss values showed a statistically significant decrease in Group 1 and Group 2 from baseline to 3 months with p = 0.001.

Plaque index

The reduction of plaque scores from baseline to 3 months post op was statistically significant in both groups with p = 0.01. Comparison between the groups at post-op visit was also statistically significant showing better outcomes in Group 2 with p = 0.0001.

Gingival index

The post op values reduced in both the groups which was statistically significant (p = 0.01). Comparison of both the groups showed statistically significant reduction of mean GI scores in Group 2 at the end of the treatment period with p = 0.0001.

Modified sulcular bleeding index

The mean values of mSBI were higher in Group 2 at baseline but post therapeutic values were more in Group 1. Statistically significant difference was found between baseline and follow-up values in both groups with p = 0.01. The difference between the groups was statistically significant in favour of Group 2 at post op visit with p = 0.0001.

Colony forming units

Colony forming units of black pigmented bacteroids on blood agar cultures were counted. The bacterial load reduced in both the groups at the end of the study period which was statistically significant and values in Group 2 which was much lower than values of Group 1 and was statistically significant (p = 0.0001).

Discussion

Scaling and root planing is one of the most commonly used procedures in treating periodontal disease and has shown to effectively decrease the microbial load and improve clinical measurements in subjects of chronic periodontitis. This is thus considered the gold standard in periodontal therapy.[9]

Of late, there have been many studies conductedevaluating the efficacy of PDT usingnumerousdyes, most common ones being methylene blue and toluidine blue O. [10]No conclusive evidence has been provided regarding their efficacy as results obtained were mostly contradictory. With this in mind, new photosensitizers such as ICG have been developed which gets activated in the absence of oxygen. In this regard, there are not many studies that explore the effects of ICG photosensitizer (AurogreenTM) as an adjunctive treatment to non-surgical periodontal treatment in the treatment of chronic periodontitis.

The results showed that both the treatment modalities led to significant improvement in all the clinical parameters (PPD, CAL, PI, GI and mSBI) and significant reduction in microbial load from baseline to three months with significant difference between groups for the parameters.

A significant reduction was noted in relation to PPD values in the test group as compared to control group, similar to the present study by A Monzavi (2016)[11]used indocyanine green as a photosensitizer for PDT along with SRP in chronic periodontitis patients. There were no significant differences between two groups at baseline. PPD showed significant improvements in the test group. Likewise, in a randomized controlled clinical trial using ICG by CP Raut et al (2018),[12] reduction was also seen in PPD and CAL in the test group as compared to control group after 6 months.

On the other hand, some studies by Pourabbas R et al[13] and Dilsiz A et al[14]contradict the PPD reduction seen in this study where it has been concluded that PDT does not have any added benefits over SRP.

Regarding Clinical Attachment Level, similar results were observed in the study by SegarraVidal et al (2017), [10]where significant gain in CAL was seen where PDT was an adjunct to SRP.Mean gain in CAL was more in PDT + SRP group,although SRP also showed significant CAL gain. Similarly, a recent systematic meta-analysis by Sgolastra F et al[15]showed that PDT when combined with SRP could improve the efficacy of treatment when compared to SRP alone in terms of attachment gain.

But there is still a bit of a controversy regarding the attachment gain that is achieved after PDT. Various studies like those by Christodoulides N et al[16]have concluded that PDT does not have any effect on the attachment gain. Some

studies have shown no significant difference in attachment gain between cases treated with SRP combined with diode laser (Borrajo JL et al[17] and Euzebio Alves VT et al[18]) or PDT (Christodoulides N et al[16]and Balata ML et al[19]) and those treated only with SRP.

With regards to PI scores, an additional benefitwas noted from PDT as an adjunctive treatment. Similar results were observed by KS Sethi et al (2019)[20] in their clinico-microbiological study that used Indocyanine green photosensitizer where significant reduction was seen in plaque index in the test group.

It is documented by Wennstrom et al[21] that treatment modalities do not affect the extent of resolution of gingivitis.Similarly, there is a lack of convincing evidence which confirms that prevention ofbiofilm formation occurs after root surface irradiation.It may be attributed tobetter oral hygiene maintenance by the subjectdue to Hawthorne effect.[22]

In this study, at the end of post-op period, statistically significant reduction in GI was observed in the test group. Similar changes in GI were reported by de Oliveria et al[23] and Yilmaz et al[24]after PDT as compared to SRP. Comparable results were also achieved by Shingnapurkar S et al (2016) [25] in their split-mouth randomized clinical trial using indocyanine green-mediated the non-surgical treatment of chronic periodontitis.

Regarding mSBI scores in the present study, the results confirm the findings of Lang et al[26]which indicate that a reduction in bleeding on probing scores is associated with reduction in periodontal inflammation. Likewise, in a randomized clinical trial by Hill G et al[27] in 2019, they used ICG for chronic periodontitis patients. The mean values for BOP decreased significantly in both groups after three months of treatment. Birang R et al[28]also reported from his study that there was reduced bleeding on probing in all groups but not with statistical significance.

Microbiological analysis to check for anaerobic bacteriawas done in the current study. A significant reduction in the colony- forming units was noted in both groups with a greater mean reduction of CFU in the test group. This is similar to a previous studydone by Srikanth K et al[29]where it was concluded that the anaerobic pathogens in PDT and laser groups showed a significant reduction of when compared to SRP group. Similarly, KS Sethi et al (2019) 20in their clinico-microbiological study used Indocyanine green photosensitizer for chronic periodontitis patients. Significant reduction of microorganisms was seen on culture of test group plaque samples in comparison with control group.

This shows a reduction in total number of anaerobic florae. It is an important determining factor of health. This could be attributed to a photothermal effect of ICG which enhanced the benefits obtained fromapplication of lasers.

ICG can get activated without oxygenin contrast to the inability of other materials (methylene blue and toluidine blue) to get activated in anaerobic environment subgingivally. Considering the limitations of the study, it can be safely concluded that ICG has a positive effect as an adjuvant to SRP in treatment of chronic periodontitis. Furthermore, it is recommended that further randomized controlled clinical trialsbe conducted with this dye, preferably with alonger study periodand a larger sample size. Its potentially beneficial effects as an adjuvant to conventional therapy can also be evaluated in some other conditions like aggressive periodontitis and peri- implantitis, to name a few.

References

1. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Laboratory investigation; a journal of technical methods and pathology. 1976 Mar;34(3):235.
2. O'Hehir TE. Going the 'full monty' in dental hygien. RDH. 1999 Aug;19(8):20.
3. Hamblin MR, O'Donnell DA, Murthy N, Contag CH, Hasan T. Rapid Control of Wound Infections by Targeted Photodynamic Therapy Monitored by In Vivo Bioluminescence Imaging. Photochemistry and photobiology. 2002 Jan;75(1):51-7.
4. GC Armitage- Development of classification system for periodontal diseases and conditions. Annals of Periodontology, 1999.

5. Grant DA, Irving B, Stern, Listgarten MA. Periodontics, 6th edition 525.
6. Carranza AF, Newman M.G Takai.H. Clinical Periodontology, 9th edition,2002,432-453.
7. Löe H. The gingival index, the plaque index and the retention index systems. The Journal of Periodontology. 1967 Nov;38(6):610-6.
8. Mombelli M. A. C. van Oosten E. Schürch Jr. N. P. Lang. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiology and Immunology 1987;2(4):145–151.
9. Lindhe, J., E. Westfelt, S. Nyman, S. S. Socransky, and A. D. Haffajee. Long- term effect of surgical/non-surgical treatment of periodontal disease: Journal of Clinical Periodontology.1984;11:448-458.
10. SegarraVidal M, Guerra‐Ojeda S, Vallés LS, Lopez‐Roldan A, Mauricio MD, Aldasoro M, et al. Effects of photodynamic therapy in periodontal treatment: A randomized, controlled clinical trial. J Clin Periodontol. 2017;44:915‐25.
11. Monzavi A, Chinipardaz Z, Mousavi M, Fekrazad R, Moslemi N, Azaripour A, Bagherpasand O, Chiniforush N. Antimicrobial photodynamic therapy using diode laser activated indocyanine green as an adjunct in the treatment of chronic periodontitis: A randomized clinical trial. Photodiagnosis and photodynamic therapy. 2016 Jun 1;14: 93-7.
12. Raut CP, Sethi KS, Kohale BR, Mamajiwala A, Warang A. Indocyanine green-mediated photothermal therapy in treatment of chronic periodontitis: A clinico-microbiological study. Journal of Indian Society of Periodontology. 2018 May;22(3):221.
13. Pourabbas R, Kashefimehr A, Rahmanpour N, Babaloo Z, Kishen A, Tenenbaum HC, Azarpazhooh A. Effects of photodynamic therapy on clinical and gingival crevicular fluid inflammatory biomarkers in chronic periodontitis: A split-mouth randomized clinical trial. Journal of periodontology. 2014 Sep;85(9):1222-9.
14. Dilsiz A, Canakci V, Aydin T. Clinical effects of potassium–titanyl–phosphate laser and photodynamic therapy on outcomes of treatment of chronic periodontitis: a randomized controlled clinical trial. Journal of periodontology. 2013 Mar;84(3):278-86.
15. Sgolastra F, Petrucci A, Gatto R, Marzo G, Monaco A. Photodynamic therapy in the treatment of chronic periodontitis: a systematic review and meta-analysis. Lasers in medical science. 2013 Feb 1;28(2):669-82.
16. Christodoulides N, Nikolidakis D, Chondros P, Becker J, Schwarz F, Rössler R, Sculean A. Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. Journal of periodontology. 2008 Sep;79(9):1638-44.
17. Borrajo JL, Varela LG, Castro GL, Rodriguez-Nunez I, Torreira MG. Diode laser (980 nm) as adjunct to scaling and root planing. Photomed Laser Surg. 2004;22(6):509-512.
18. Euzebio Alves VT, de Andrade AK, Toaliar JM, et al. Clinical and microbiological evaluation of high intensity diode laser adjutant to non-surgical periodontal treatment: a 6-month clinical trial. Clin Oral Investig. 2013;17(1):87-95.
19. Balata ML, Andrade LP, Santos DB, Cavalcanti AN, Tunes UD, Ribeiro ED, Bittencourt S. Photodynamic therapy associated with full-mouth ultrasonic debridement in the treatment of severe chronic periodontitis: a randomized-controlled clinical trial. Journal of Applied Oral Science. 2013 Apr;21(2):208-14.
20. Sethi KS, Raut CP. Antimicrobial photodynamic therapy using indocyanine green as a photosensitizer in treatment of chronic periodontitis: A clinico-microbial study. Indian Journal of Dental Research. 2019 Nov 1;30(6):870.
21. Wennstrom JL, Heijl L, Lindhe J. Periodontal Surgery: Access Therapy. Bangalore, Karnataka: Blackwell Munksgaard; 2008: 783-822. Clinical Periodontology and Implant dentistry; vol 1.
22. Feil PH, Grauer JS, Gadbury‐Amyot CC, Kula K, McCunniff MD. Intentional use of the Hawthorne effect to improve oral hygiene compliance in orthodontic patients. J Dent Educ 2002;66: 1129‐35.

23. de Oliveira RR, Schwartz-Filho HO, Novaes Jr AB, Taba Jr M. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: A preliminary randomized controlled clinical study. Journal of periodontology. 2007 Jun;78(6):965-73.
24. Yilmaz S, Kuru I, Kuru L, Noyan U, Argun D, Kadir T. Effect of gallium arsenide diode laser on human periodontal diseases; a microbiological and clinical study. Lasers Surg Med. 2002;30: 60–6.
25. Shingnapurkar S, Mitra D, Kadav M, Shah R, Rodrigues S, Prithyani S. The effect of indocyanine green-mediated photodynamic therapy as an adjunct to scaling and root planing in the treatment of chronic periodontitis: A comparative split-mouth randomized clinical trial. Indian Journal of Dental Research. 2016 Nov 1;27(6).
26. Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease? J Clin Periodontol.1986;13(6):590-596.
27. Hill G, Dehn C, Hinze AV, Frentzen M, Meister J. Indocyanine green-based adjunctive antimicrobial photodynamic therapy for treating chronic periodontitis: A randomized clinical trial. Photodiagnosis and photodynamic therapy. 2019 Jun 1;26:29-35.
28. Birang R, Shahaboui M, Kiani S, Shadmehr E, Naghsh N. Effect of nonsurgical periodontal treatment combined with diode laser or photodynamic therapy on chronic periodontitis: a randomized controlled split-mouth clinical trial. Journal of lasers in medical sciences. 2015;6(3):112.
29. Srikanth K, Chandra RV, Reddy AA, Reddy BH, Reddy C, Naveen A, et al. Effect of a single session of antimicrobial photodynamic therapy using indocyanine green in the treatment of chronic periodontitis: A randomized controlled pilot trial. Quintessence Int 2015;46: 391‐400.