Journal of the International Academy of Periodontology (2023) **25/4**:208-218

DOI: https://doi.org/10.14436/1466-2094.25.4.208-218.oar

Effects of laser and indocyanine-green mediated antimicrobial photodynamic therapy on the red-complex bacteria and crevicular procalcitonin level in periodontitis patients: A split-mouth randomized clinical trial

Anil Arya[1], Sridharan Srirangarajan[1], Ravi Rao[1], Srikumar Prabhu[1], Oradiyath Deepika[1]

[1]Bangalore Institute of Dental Sciences and Post-Graduate Research Center, Department of Periodontics (Bangalore, India).

Abstract

Objective: To determine the effects of laser and photodynamic therapy (PDT) on crevicular procalcitonin (PCT) and red-complex bacteria in periodontitis stage II grade A patients.

Methods: 66 sites with periodontitis were selected. After scaling and root planing (SRP), the participants were randomized to receive 810-nm diode laser and PDT using indocyanine green (ICG). The change in the crevicular PCT levels and red-complex bacterial loads using BAPNA assay was the primary outcome, probing depth (PPD), clinical attachment loss (CAL), relative attachment level (RAL), plaque and gingival indices were the secondary outcome, measured at baseline and 3 month post-intervention.

Results: Within each group, significant improvements (P<0.001) were found for all variables in 3 month follow-up, compared to the baseline. Of the treatment groups, SRP+ICG-PDT showed a better reduction in the BAPNA and PCT levels than SRP+ laser and SRP alone. Correlation analysis between BAPNA and PCT showed a significant positive correlation (r= 0.64; p=0.001).

Conclusion: In periodontitis stage II grade A patients, a single application of PDT (using 810-nm laser and ICG) provided enhanced benefit compared to SRP alone or SRP + laser, in terms of reduction of red complex bacterial load and gingival crevicular procalcitonin levels 3 months following the intervention.

Keywords: Procalcitonin. Laser. Photodynamic therapy. Periodontitis. Scaling and root planing. Gingival crevicular fluid. Red-complex bacteria.

Introduction

Periodontitis is one of the major causes of tooth loss in adults, and is considered primarily an anaerobic bacterial infection caused by the red complex species. Enzymes, endotoxins, and other cytotoxic factors from these bacteria lead to tissue destruction and initiate chronic inflammation (Page and Kornman, 1997). Debridement in the form of scaling and root planing (SRP) is the most commonly employed form of mechanical therapy. It is used not only to treat periodontal diseases, but is also used to maintain the periodontium after surgical therapy and in the prevention of disease recurrence (Orlandi *et al*., 2022). Periodic mechanical disruption of oral microbial biofilm or maintaining therapeutic concentrations of antimicrobials in the oral cavity are full of limitations (Pretzl *et al*., 2019; Socransky and Haffajee, 2002), hence suitable adjuncts to SRP need to be investigated. Combining SRP with laser disinfection or photodynamic therapy (PDT) has been shown to improve the antimicrobial effects, and this seems to be important, as an increasing number in antibiotic resistance has been documented for a vast number of periodontopathic microorganisms (Pretzl *et al*., 2019; Khattri, *et al*., 2020, Feres *et al*., 1999). Lasers generate unipolar compression waves, which are one of the latest technology platforms for killing biofilm microorganisms at a shorter time (Amaroli A *et al*., 2022). Apart from being antimicrobial, lasers have cell proliferative, analgesic, and anti-inflammatory effects (Doukas *et al*., 1996). The reduction of prostaglandin E2 (PG-E2) by laser application has been considered very useful in limiting the progression of gingivitis or periodontitis (Aoki *et al*., 2004).

Correspondence to: Sridharan Srirangarajan
E-mail: docranga@yahoo.com

However, its effectiveness in the treatment of periodontal disease is still controversial, and the concept of PDT came through. In PDT the photosensitizing agent is excited to a higher energy state in the presence of oxygen, by utilizing the laser light energy to release singlet oxygen, which is cytotoxic to bacteria (Amaroli *et al*., 2022). It is interesting to note that the radius of action cannot usually exceed more than 0.02μm, and the primitive molecular nature of singlet oxygen limits the microorganisms to develop any resistance to this cytotoxic reaction (Konopka, *et al*., 2007 and George *et al*., 2009).

There is no consensus in the literature to show greater clinical benefits of PDT as an adjunct to SRP than the results of SRP alone (Giannopoulou *et al*., 2012, Jia *et al*., 2020 and Christodoulides *et al*., 2008). It is possible that short exposures to light or the photo-sensitizer used may be responsible for the lack of clinical benefits. (Merchat *et al*., 1996 and Soukos *et al*., 2011).

Indocyanine green (ICG) has shown promising usage in the medical diagnostics relating to heart, liver, and ophthalmic diagnosis, and has been recently introduced in dentistry (Bashir *et al*., 2021). The key features making its use in periodontics worthwhile are its effectiveness even in areas with no oxygen, its rapid uptake by anaerobic bacteria, and non- staining of the tissues in the vicinity of usage (Marshall *et al*., 2010). The wavelength of laser light for activation of ICG also concurs to the commonly used soft tissue diode lasers in the field of non-surgical periodontal therapy. Among several markers for inflammation and sepsis, procalcitonin (PCT) and C reactive protein (CRP) are being studied for their accuracy in the diagnosis of bacterial infection. But after SRP, the levels of CRP were reduced within the normal range, reflecting its limitation to predict the residual bacterial infection or inflammation (Srirangarajan *et al*., 2022). Serum PCT has been shown to be a better marker for predicting bacteremia and residual infection after SRP (Ranjitha *et al*., 2021). With this background, we hypothesize that ICG-mediated PDT would reduce the red complex bacterial load and subsequent gingival crevicular fluid (GCF) PCT levels more than SRP alone or SRP plus laser in stage II grade A periodontitis patients. Therefore this prospective, split-mouth, double-blind, parallel designed randomized controlled clinical trial was envisioned to compare and evaluate the efficacy of diode laser and ICG mediated photodynamic therapy and SRP on the red complex microflora, and the associated changes in the gingival crevicular procalcitonin levels in stage II grade A periodontitis patients.

Materials and Methods

Study design and registration

The present study was a prospective double-blind, parallel design, split-mouth, randomized, clinical trial conducted at Bangalore Institute of Dental Sciences and Post-Graduate research center (BIDS), from September 2022 to February 2023. The study was approved by the ethical committee of BIDS and was designed and conducted following the declaration of Helsinki as modified in 2013. This trial evaluated the 3 months clinical, microbiological, and biochemical outcomes of periodontal pocket treatment by SRP alone, SRP plus laser, and SRP plus PDT using ICG, similar to the reports in literature (Lindhe J *et al*., 1982). The primary outcome was reduction in microbial and PCT values at the end of 3 months. The secondary outcomes were: changes in the probing pocket depth (PPD), clinical attachment loss (CAL), relative attachment level (RAL), gingival index (GI), and plaque index (PI). This study followed the consolidated standards of reporting (CONSORT) trials guidelines (Fig 1), and was registered in the clinical trial registry India under the identifier CTRI/2022/08/045099, before the start of the patient's enrollment.

Sample size calculation

Sample size calculation was done by using nMaster version 2.0 software (Christian Medical College; Vellore, India). The effective sample size for the present clinical trial was estimated by applying a power of 85% and α-error set at 5%. Sixty sites from 20 participants would be needed to detect statistically significant differences. Considering a 10% attrition rate during the 3 months follow-up period, 3 sites were increased in each group from 2 patients, making it a total of 66 periodontal pocket sites from 22 patients. Considering the mean and standard deviation from the data acquired, the statistical effect size (Cohen's d) was calculated with deviation of 1.4 between the groups.

Examiner calibration

A single examiner (AA) was calibrated before the study. Five subjects who were not included in the study were requested to volunteer for the calibration exercise. The clinical parameters recording was done in two sessions 48 hours apart. Intra-examiner calibration was performed by assessing PPD in duplicate. The periodontal parameters estimation was judged to be reproducible if the kappa value (0.8) for the calibration exercise showed good agreement for observation and measurement.

Study participants

Twenty-two patients aged between 35-55 years with a diagnosis of Stage II grade A periodontitis, according to the new classification (Papapanou *et al* 2018) and case definition of periodontitis were recruited They had radiographic evidence of bone loss extending to the coronal third (15-33%), inter-dental CAL of 3-4 mm, with no tooth loss due to periodontitis, maximum PPD of 5 mm, with mostly horizontal bone loss. Patients were included if had: (i) ASA stage 1- good general health;

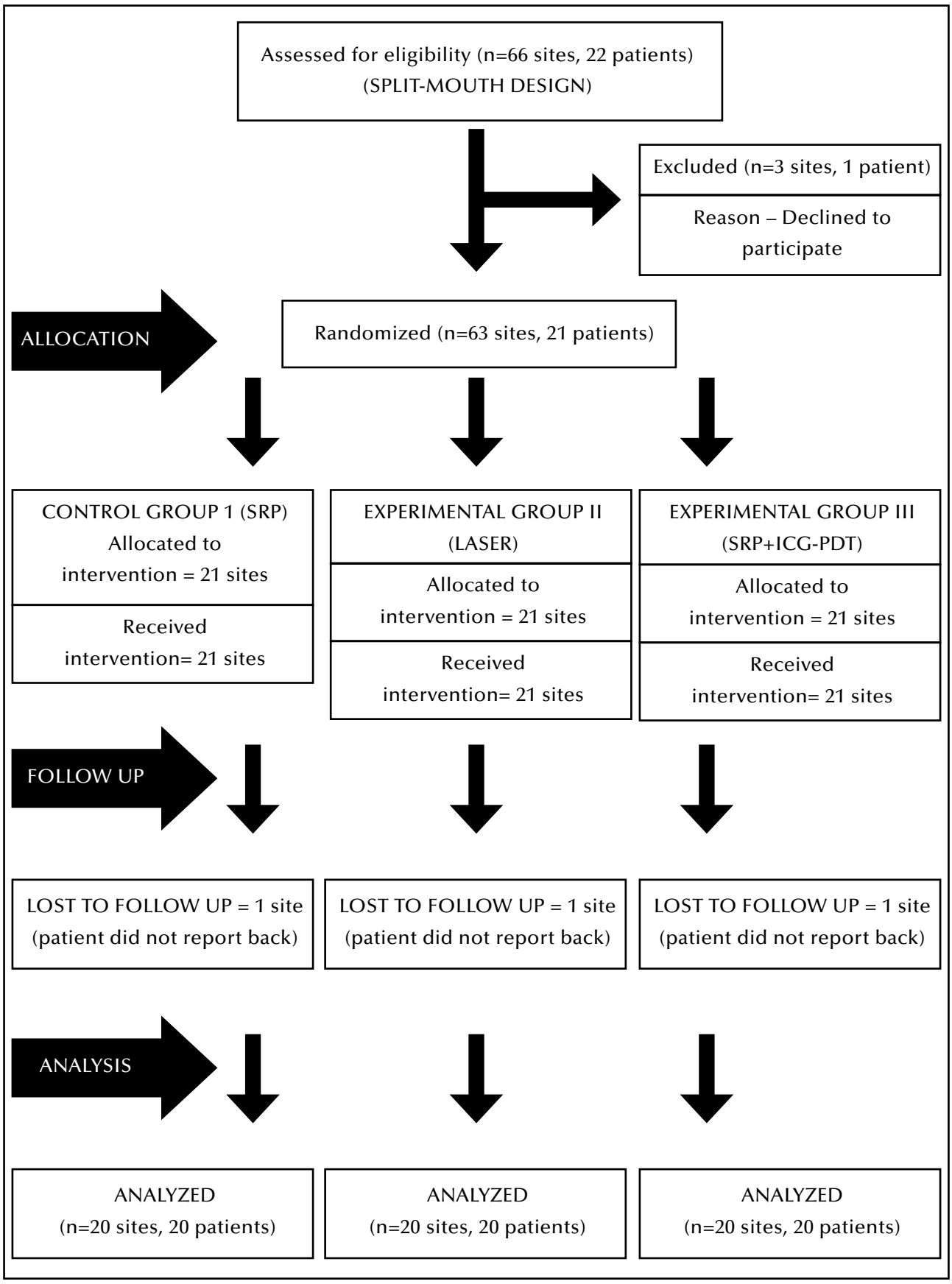


Figure 1. CONSORT flow diagram.

(ii) the presence of 3 multi-rooted teeth in the posterior segment with identical PD ≤ 5 mm (one in each quadrant); (iii) more than 20 permanent teeth excluding third molars. Patients with the following criteria were excluded: (i) known allergic to ICG dye; (ii) those who had received antibiotics, or surgical or non-surgical periodontal treatment within the past 12 months; (iii) pregnant patients; (iv) current smokers; (v) history of treatment for recent bacterial infections; (vi) thyroid disorders; (vii) evidence of other systemic diseases (ischemic heart disease, liver disease, renal disease, immunodeficiency, and diabetic mellitus); (viii) HIV, hepatitis or any other contagious diseases.

The treatment and follow-up periods were explained verbally to the patients, and comprehensive oral hygiene instructions were given. Only those who agreed to participate signed a written informed consent.

Clinical periodontal parameters data collection

The clinical periodontal measurements were performed by one calibrated examiner who was blinded to the experimental sites. The following information was recorded: full-mouth plaque index scores; full-mouth gingival index scores (Loe and Silness, 1963). PPD (mm), measured from the gingival margin to the base of the periodontal pocket; and interdental CAL (mm), measured from the inter-dental cement enamel junction (CEJ) to the base of the periodontal pocket, were both measured using a manual probe (UNC-15-HuFriedy, Chicago, USA) at six sites per tooth surface (mesio-facial, facial, disto-facial, mesiolingual lingual, distolingual). The deepest PPD and inter-dental CAL of the examined tooth were registered. RAL (mm) was measured for the sites to be used for treatment, using a customized acrylic stent, from a fixed reference point to the base of the periodontal pocket. All the clinical measurements (Listagarten, 1980, Yang *et al*., 1992) were done at baseline (before the treatment) and 3 months post-treatment.

Randomization and grouping of patients

Identical sites, one in each arch, on the multi-rooted teeth were randomly allocated to the intervention and control groups at an allocation ratio of 1:1, using a computer-guided allotment software (CLINSTAT MS DOS; United Kingdom), as follows:

» Group I: SRP alone (control) – 22 sites;

» Group II: SRP + LASER (810-nm diode laser) – 22 sites;

» Group III: SRP + PDT (ICG mediated PDT using 810-nm diode laser) – 22 sites.

Microbiome and gingival crevicular fluid (GCF) sample collection

Procedure for subgingival plaque sample

After the site selection and clinical examinations, supragingival plaque was carefully removed using hand and ultrasonic scaling instruments (Woodpecker, China). Subgingival plaque sample was then collected, using sterile Gracey curettes (HuFriedy, Chicago, USA). After isolating the area, the curette was inserted parallel to the long axis of the tooth into the deepest portion of the periodontal pocket. It was then moved coronally by scraping along the root surface. The collected samples were stored in 1ml of Brucella Broth solution contained in micro-centrifuged polypropylene vials (1.5ml with conical bottom, Digital Hub®, India). The samples were then dispatched to the laboratory on the same day and stored at -20°C until further analysis for estimation of the red complex microorganisms. The same procedure was repeated at the end of 3 months after completion of the treatment.

Procedure of GCF collection

GCF samples were collected for the allocated sites a day after the collection of plaque samples and recording of clinical parameters. The selected sites were air-dried and isolated using cotton rolls, to avoid blood and saliva contamination. GCF was collected by calibrated microcapillary pipettes (Global Scientific, India) of known diameter. The calibrated pipettes were placed at the entrance of the gingival crevice of the selected tooth and a standardized volume of 2-3 µl of GCF was collected and immediately transferred to plastic vials (Digital Hub®, India) containing Phosphate Buffer Saline (PBS). It was then stored at -20°C until further analysis for procalcitonin by ELISA. The same sampling procedure was followed at the end of 3 months after the completion of treatment.

Treatment

The selected sites from each individual were then randomly allocated to the therapeutic groups by a dental hygienist (DH). The interventions were then entirely performed by an experienced periodontist (BT) who was unaware of the main objectives of the study and data collection.

Protocol for nonsurgical periodontal therapy (NSPT)

All participants (22) received full mouth supra and subgingival scaling and root planing using a combination of manual and ultrasonic instruments. Two-stage protocols at a gap of 24 hrs were followed for the same. For one of the three selected treatment group, SRP alone was done. The remaining two sites in the split-mouth design received Laser and PDT in addition to SRP.

Laser therapy and antimicrobial PDT

Twenty-two sites were selected for laser therapy. A diode laser (ZOLAR Technology; Ontario: CANADA) with a wavelength of 810 nm, and 0.8W output in continuous mode was chosen for pocket disinfection/sulcular debridement. The fiber tip (400 microns), which was placed at the deepest portion of the pocket and the laser, was activated by pressing the footswitch. The tip was moved using an inward and outward movement, parallel to the long axis of the tooth, within the gingival sulcus, for 60 seconds. For the remaining 22 sites receiving PDT, the periodontal pocket was filled with a fresh solution, by mixing 5mL of sterile water to 5mg/mL of the ICG stock solution. The stock solution was further diluted in a 1:5 ratio, to achieve a final concentration of 5mg/ml. The delivery of the photosensitizer into the pocket was done using a flexible applicator tip (0.04 mm PACT Curndente) in a coronal direction, starting from the most apical portion. A photosensitization period of 60 sec was given and then the pockets were rinsed thoroughly with distilled water, and the sites were irradiated for 60 sec using the same wavelength laser and protocol as explained in the laser treatment group.

Follow-up visits

All the treated patients were monitored by the dental hygienist for compliance with oral hygiene instructions given, and were instructed to refrain from any other forms of oral hygiene aids, like dental floss or the use of mouthwash. There were no reports of any adverse events throughout the study period. The subjects were recalled at the end of 3 months, and the clinical, microbiological, and the biochemical tests were repeated in a similar way to the baseline records.

Laboratory procedures

Quantification of red complex bacteria using Nα-Benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA) assay

One milliliter of a solution containing the enzyme-substrate BAPNA (Sigma Aldrich B-4875) with a final concentration of 1.0 nmol/l in the assay buffer (0.05 mmol/l Tris-HCl, 5 mM $CaCl_2$, pH 7.5), containing 5% dimethyl sulfoxide (DMSO), was added to the Eppendorf tubes containing plaque samples. This suspension was vortexed and then placed in an ultrasonic bath on ice for 10 minutes with 2-second cycles and 2-second intervals, at 17 W using a 100-W ultrasonic processor. After 30 minutes of incubation at 37°C, the reaction was stopped by the addition of 100 ml glacial acetic acid. A spectrometric assay was performed (OD 405nm) and, as a measure of protease activity, the color changed from clear to yellow. The quantitative calculation for red-complex was done based on the OD values.

GCF analysis for procalcitonin, by enzyme-linked immunosorbent assay (ELISA)

Human Procalcitonin ELISA kit (Evron life sciences, India), with a sensitivity of - 0.00249ng/ml and range of 0.005ng/ml – 2ng/ml, was used for this study. All reagents were brought to room temperature before use. Standard reconstitute: 120µl of the standard (2.4ng/ml) with 120µl of standard diluent was used to generate a 1.2ng/ml standard stock solution, as per the instructions given. 40µl sample was added to the corresponding sample wells and then 10µl anti-PCT antibodies were added to sample wells. 50µl streptavidin-HRP (horseradish peroxidase) was added to both the sample wells and standard wells, and mixed. It was then incubated for 60 minutes at 37°C. After removing the sealer, the plates were washed with wash buffer (300 µl/well) for 5 times. After washing, 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. The plate was covered and re-incubated for 10 minutes at 37°C in the dark. 50µl Stop Solution was added to each well, and the optical density (OD) values of each well were determined immediately, using a microplate reader set to 450 nm within 10 minutes.

Statistical analysis

All statistical analyses were computed using R version 4.2.2 software (Bell Laboratories, University of Auckland). Normality of data was checked using Shapiro-Wilk test and Q-Q plotting. Descriptive statistics such as mean, standard deviation (SD), and range values was calculated for variables in normal distribution. Intra-group comparison of the full-mouth indices was done using repeated measures ANOVA. Intra-group comparison of the primary and secondary outcome variables was done using paired *t*-test. Inter-group comparison was done using unpaired *t*-test. To correlate the red complex microorganism counts to procalcitonin, Pearson's correlation analysis was done. Statistical significance was considered if the *p*-value was less than 0.05.

Results

Of the 22 patients (66 sites) treated for periodontitis, one patient (3 sites) was lost during the follow-up. Thus a total of 21 patients (63 sites) were subjected to statistical evaluation. The mean age of the patients was 40.7 ± 7.19 years (7 females and 14 males) given a total of 63 teeth, with an equal number of 21 sites for each of the three treatment groups. The intra-group comparison using repeated measures ANOVA showed significant reduction in both mean plaque scores (1.48±0.16 to 1.01±0.14) and gingival scores (1.38±0.18 to 0.93±0.21) at 3 months than at baseline (p <0.001) (Table 1). Intra-group analysis by paired *t*-test showed significant reduction in both the primary outcome measures (red-complex bacteria and GCF PCT) at three

months post-treatment for all the treatment groups (p<0.001) (Table 2). Inter-group comparative results using *t*-test showed PDT to reduce both BAPNA and PCT better than the laser and SRP groups (MD for BAPNA 1.33 and MD for PCT 0.14 ng/ml) (Table 3, Fig 2). Intra-group analysis by paired *t*-test showed all three treatment groups to significantly show reduction in PPD (p < 0.001), with higher reduction in CAL and RAL for the laser group, followed by PDT and SRP (p < 0.001) (Table 4). Inter-group analysis for PPD CAL and RAL at 3 months using unpaired *t*-test showed a higher reduction in PPD for the PDT group, compared to laser and SRP, although insignificant between the groups. Both laser and PDT showed a significant reduction in comparison to SRP alone (p =0.01), CAL showed higher CAL gain in laser group followed by PDT and SRP groups, but the differences between all three groups were not statistically significant (p =0.91). There was higher RAL gain in the PDT group, followed by laser and SRP, but the differences between PDT and laser were statistically insignificant, whereas the differences between PDT and SRP as well as laser and SRP were statistically significant (p =0.003) (Table 5, Fig 3). Pearson's correlation statistics revealed a mild to moderate positive significant correlation between BAPNA and PCT at 3 months (p< 0.001) (Fig 4).

Table 1. Comparative analysis of plaque and gingival index scores by repeated measures ANOVA.

Variable	Baseline	3 month	*P value**
GI (mean ± SD)	1.38±0.18	0.93±0.21	< 0.001
PI (Mean ± SD)	1.48±0.16	1.01±0.14	< 0.001

GI - gingival index; PI - Plaque index.

Table 2. Intra-group comparison of primary outcome variables within groups, by paired *t*-test.

	BAPNA				PCT			
Group	Baseline	3 months	MD	*P* value	Baseline	3 months	MD	*P* value
SRP	1.64±0.22	1.33±0.23	0.31	<0.001	0.20±0.03	0.12±0.01	0.08	<0.001
Laser	1.64±0.21	0.84±0.39	0.8	<0.001	0.20±0.01	0.11±0.01	0.09	<0.001
PDT	1.67±0.20	0.34±0.32	1.33	<0.001	0.20±0.01	0.06±0.01	0.14	<0.001

SRP - Scaling and root planing group; PDT - Photodynamic therapy group; MD - mean difference; BAPNA - Nα-Benzoyl-DL-arginine p-nitroanilide hydrochloride assay; PCT - procalcitonin.

Table 3. Inter-group comparison of primary outcome variables at 3 month, using unpaired *t*-test.

GROUP	BAPNA	PCT
SRP	1.33 ± 0.23[a]	0.12 ± 0.01[a]
Laser	0.84 ± 0.39[b]	0.11 ± 0.01[b]
PDT	0.34 ± 0.32[c]	0.06` ± 0.01[c]
P-value	< 0.001	< 0.001

SRP - Scaling and root planing group; PDT - Photodynamic therapy group; BAPNA - Nα-Benzoyl-DL-arginine p-nitroanilide hydrochloride assay; PCT - procalcitonin.

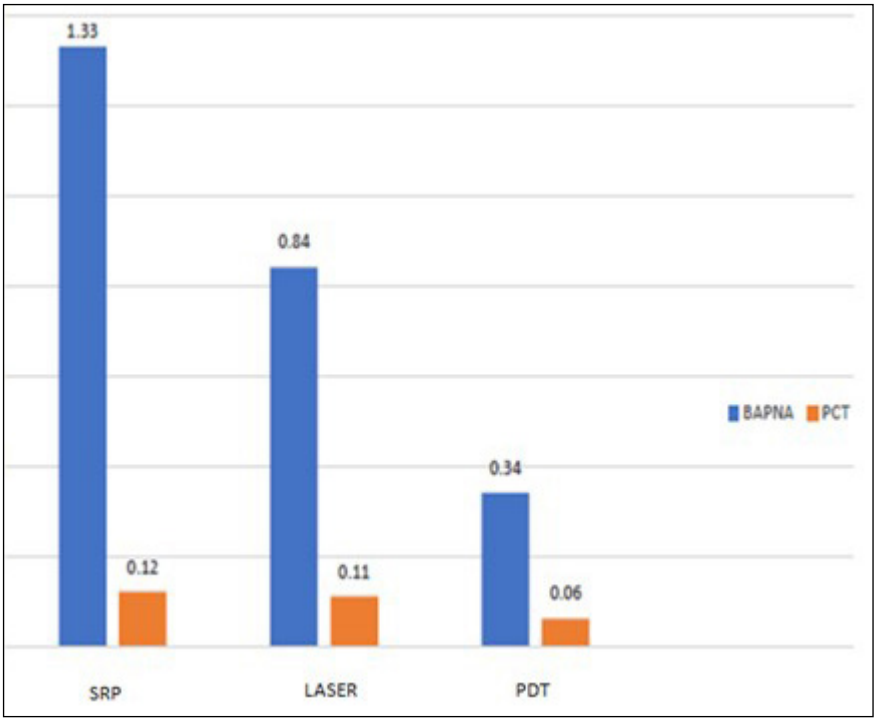


Figure 2. Inter-group comparison of primary outcome variables at 3 month, using unpaired t-test.

Table 4. Intra-group comparison of secondary outcome variables, by paired *t*-test.

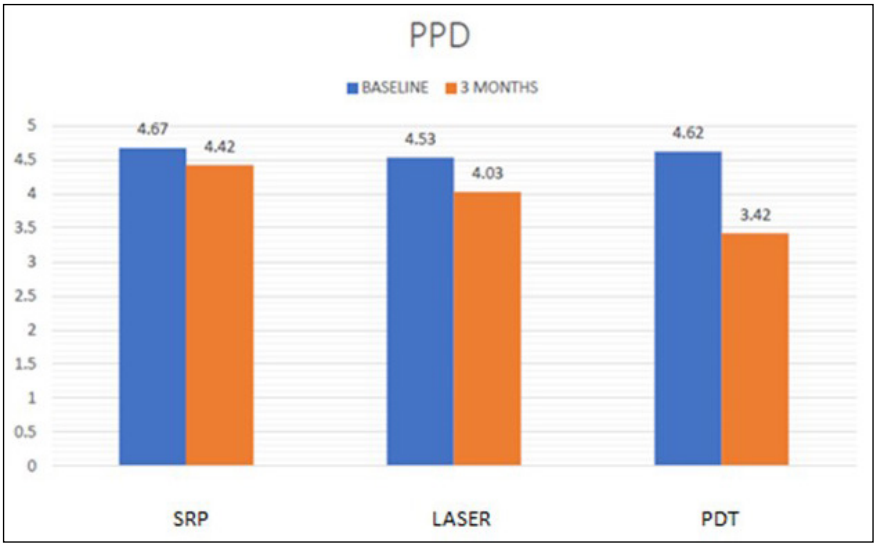
	Groups	Baseline	3 Month	MD	*P*-Value
PPD (mm)	SRP	4.67 ± 0.64	4.42 ± 0.67	0.25	0.001*
	Laser	4.53 ± 0.55	4.03 ± 0.50	0.5	<0.001*
	PDT	4.62 ± 0.58	3.97 ± 0.43	0.65	<0.001*
CAL (mm)	SRP	4.05 ± 0.90	3.62 ± 1.03	0.42	0.013*
	Laser	4.19 ± 0.85	3.65 ± 0.64	0.54	<0.001*
	PDT	4.24 ± 0.98	3.73 ± 0.88	0.52	<0.001*
RAL (mm)	SRP	8.05 ± 0.82	7.56 ± 1.20	0.49	0.022*
	Laser	7.99 ± 0.71	6.88 ± 0.52	1.11	<0.001*
	PDT	7.94 ± 0.88	6.69 ± 0.60	1.25	<0.001*

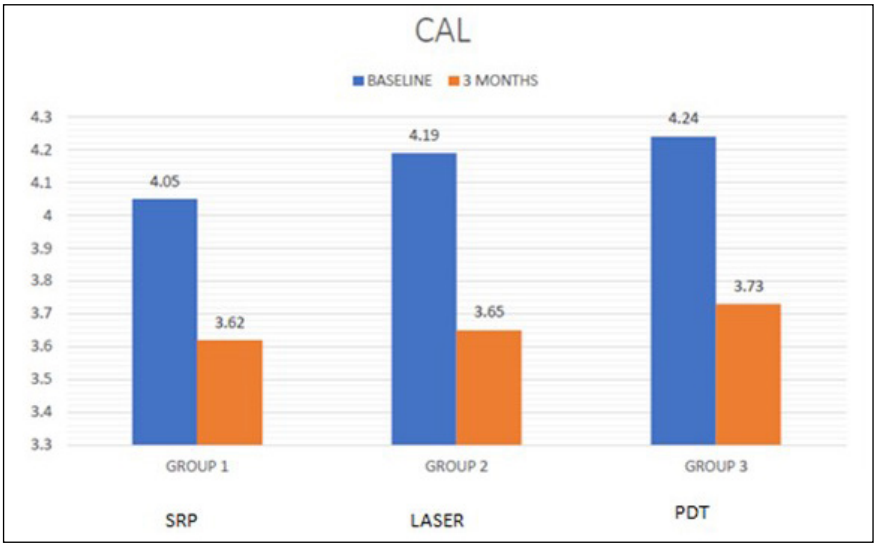
SRP - Scaling and root planing group; PDT - photodynamic therapy group; MD - Mean difference; PPD - Probing pocket depth; CAL - Clinical attachment loss; RAL - Relative attachment level.

Table 5. Inter-group comparison of secondary outcome variables at 3 months, by unpaired *t*-test.

Group	PPD		CAL		RAL	
	Baseline	3 Months	Baseline	3 Months	Baseline	3 Months
SRP	4.67± 0.6	4.42 ±0.62[a]	4.05 ±0.9	3.62± 1.03[a]	8.05 ±0.82	7.56 ±1.20[a]
Laser	4.53± 0.5	4.03 ±0.45[b]	4.19 ±0.85	3.65± 0.64[a]	7.99± 0.71	6.88 ±0.58[b]
PDT	4.62 ±0.58	3.42± 0.43[b]	4.24 ±0.98	3.73± 0.8[a]	7.94± 0.88	6.69± 0.5[b]
P value	0.72	0.01	0.78	0.91	1.000	0.003

SRP - Scaling and root planing group; PDT - photodynamic therapy group; MD - Mean difference; PPD - Probing pocket depth; CAL - Clinical attachment loss; RAL - Relative attachment level.





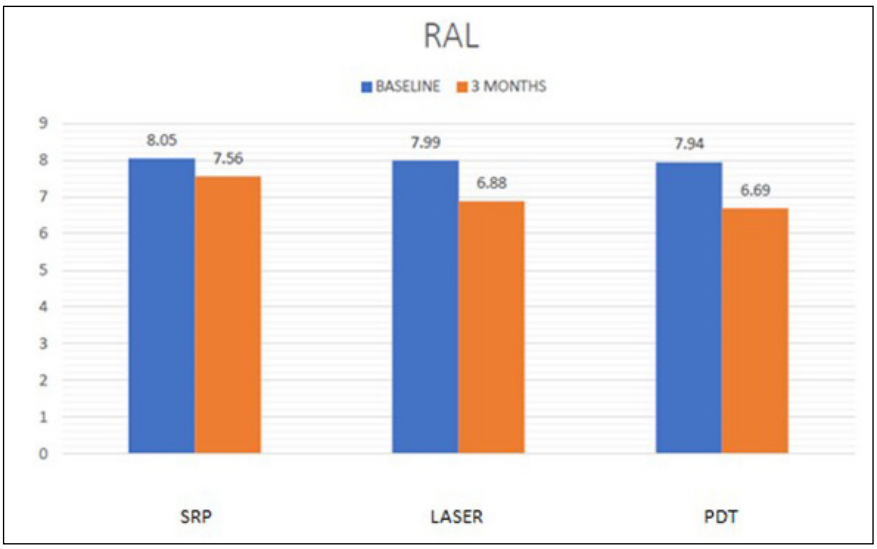


Figure 3. Inter-group comparison of clinical parameters.

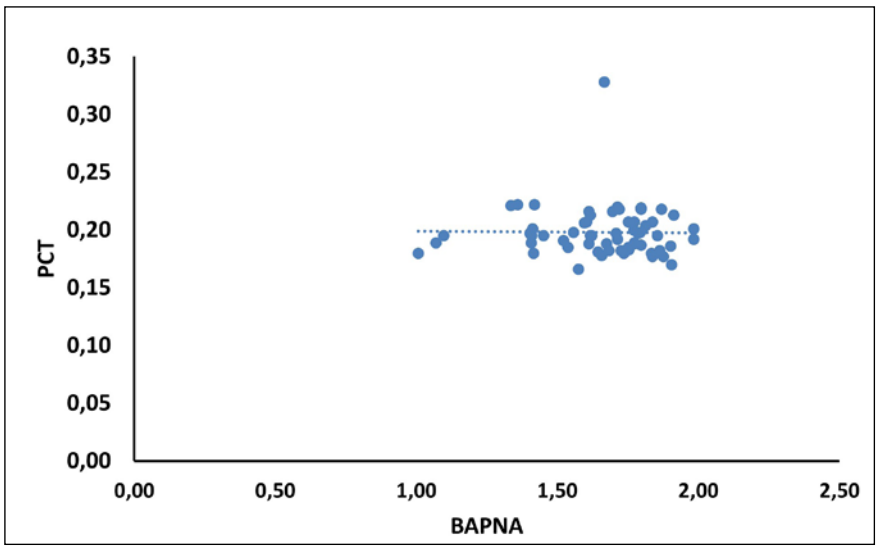


Figure 4. Correlation between PCT and BAPNA.

Discussion

In the present study, it was found that all three treatment groups demonstrated a significant reduction in red complex bacterial counts, which was reflected by their crevicular PCT levels as well. Inter-group comparisons showed statistically significant reductions, with superior outcomes for the microbial reduction in the ICG-PDT (MD=1.33) group, followed by the laser group (MD=0.8) and SRP group (MD=0.31).

On a biological level, it is known that the percentage of red complex species of bacteria is 0.15% of the total bacteria counts in periodontal health, and attains three folds the numbers in deeper pockets (>3mm). Controversial results on the antimicrobial efficacy of both laser and PDT in periodontal pockets speculate its benefits over conventional SRP (Haffajee *et al*.,1995, Xue *et al*., 2017, Azarpazhooh *et al*., 2010). The cause for these biased results could be the presence of bivalent cations in the anaerobic environment of the periodontal pocket, which can limit or reduce the uptake of various photosensitizers like the Methylene blue, Toluidine blue O, Chlorine e6, which were used in a majority of these studies (Alwaeli *et al*., 2015). The laser group also showed a significant reduction in the red complex bacterial counts, which could be attributed to the wavelength (810nm) and its excellent tissue ablation and detoxifying properties (De Sousa *et al*., 2016). The results of the current study are in agreement with the conclusion of the systematic review done by Qadri *et al*. (2015), which stated that diode lasers of wavelength 808-980nm displayed an effective reduction in the gram-negative anaerobic periodontal pathogens in pockets ranging from 5-6mm depth. Although we know that SRP can be clinically successful, it may not eradicate all the putative pathogens. The persistence or re-growth of these microorganisms should be considered a cause for unsatisfactory treatment outcomes. This was also evidenced in our study when SRP was compared to PDT and laser group for reduction of red complex microbial load. It would be of interest to note that bacterial counts returned to near pre-treatment levels within 3-7 days after SRP, but there was a predominant shift from gram-negative to a gram-positive subgingival microflora, which persisted for an extended duration (Slots *et al*., 1979). This could probably be an explanation as to why we got a significant reduction in red complex microbial loads at the end of 3 months even in the SRP group.

A meta-analysis report shows PCT as a promising biomarker for bacterial infection (Simon *et al*., 2004). Studies on PCT in periodontal disease have shown PCT to be useful to predict the severity of periodontal disease and also to be sensitive enough to relate the transient bacteremic changes after non-surgical periodontal therapy (Srirangarajan *et al*., 2022, Ranjitha *et al*., 2021). The GCF PCT values in our study ranged from 0.17 ng/ml to 0.32 ng/ml in three months after SRP, and after adjunctive periodontal therapy these values ranged from 0.04 ng/ml to 0.13 ng/ml. The inter-group comparison displayed a significant reduction in the PDT group (0.06ng/ml), followed by the laser group (0.11ng/ml) and SRP group (0.12ng.ml). A positive correlation was seen between BAPNA and PCT levels at both baseline and 3 months. These findings suggest that PCT levels may tend to increase or decrease in conjunction with red complex bacterial levels, supporting its hypothetic correlation to microbial infection. The results of our study are in accordance with the limited literature available (Giannopoulou *et al*., 2012, Selvadurai *et al*., 2019).

The results of clinical parameters revealed a significant improvement in probing pocket depth (PPD) (MD=0.65mm), CAL gain (MD=0.52mm) and RAL gain (MD=1.25mm) in the ICG-PDT group, followed by the laser group and SRP group at 3 months. The observed benefits in the clinical periodontal parameters

can be attributed to the positive effects of SRP (Slots *et al*., 1979, Lindhe J *et al*., 1982). Studies showed that the adjunctive use of PDT with SRP may confer additional benefits in terms of clinical periodontal parameters (Christodoulides *et al*., 2008, Soukos *et al*., 2011, Bashir *et al*., 2021). Observing the previous reports, it could be inferred that both SRP and laser could reduce PPD by 1mm and CAL by 0.5mm at the end of 3 months (Cobb, 2002, Mills *et al*., 2019), whereas PDT exhibited a decrease of 1.17mm in PPD and CAL gain of 0.07mm (Bashir *et al*., 2021) contradicting the report by Hill *et al*. (2019), wherein they found no effect of PDT on attachment gain.

The irradiation time needed for the significant inactivation of bacteria in a biofilm model was found to be longer and varies from the 60s to even 5 min (Xue *et al*., 2017). In this study, only a single episode of PDT was performed for 60s per tooth, also the dose of photosensitization in combination with the laser power density and its acting time in the tissues and oxygen could have influenced the clinical outcome in our study. No patients reported any adverse effects or staining of the teeth and surrounding oral tissues during the treatment. Significant reduction in the full-mouth GI and PI scores in all the three treatment groups could indicate an improved compliance. The present study employed a randomized controlled split-mouth design, which minimizes inter-subject variability and potentially requires fewer subjects, although it is important to select patients with a symmetrical disorder (Deaton., 2010, Lesaffre *et al*., 2009). Further investigations using a larger sample size with different stages of periodontal disease are needed to validate the results and confirm the efficacy of ICG-PDT.

Conclusion

Newer adjunctive modes of periodontal therapy have stressed the need for greater reduction in bacterial counts, which has a profound effect on the clinical results. In this view, the results of our study indicate superior benefits of using ICG-PDT, in comparison to laser and SRP, in the reduction of red-complex bacterial counts. Crevicular PCT has proven to be an excellent indicator for this reduction of bacterial counts.

References

Aoki A, Sasaki KM, Watanabe H and Ishikawa I. Lasers in nonsurgical periodontal therapy. *Periodontol 2000* 2004; **36**: 59-97.

Amaroli A, Ravera S, Zekiy A, Benedicenti S., and Pasquale, C. A narrative review on oral and periodontal bacteria microbiota photobiomodulation, through visible and near-infrared light: from the origins to modern therapies. *Int J Mol Sci*. 2022; 25: **23**(3):1372.

Azarpazhooh A, Shah PS, Tenenbaum HC, Goldberg MB. The effect of photodynamic therapy for periodontitis: A systematic review and meta-analysis. *J Periodontol.* 2010; **81**: 4-14.

Alwaeli HA, Al-Khateeb SN, Al-Sadi A. Long-term clinical effect of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. *Lasers in medical science*. 2015; **30**: 801-7.

Bashir NZ, Singh HA, Virdee SS. Indocyanine green–mediated antimicrobial photodynamic therapy as an adjunct to periodontal therapy: A systematic review and meta-analysis. *Clin Oral Invest.* 2021; **25**: 5699-710.

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. *J Periodontol.* 2008; **79**: 1638-44.

Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. *J Clin Periodontol* 2002; **29**: 22-32.

Doukas AG, Flotte TJ. Physical characteristics and biological effects of laser induced stress waves. *Ultrasound Med Biol* 1996; **22**, 151-164.

De Sousa NT, Gomes RC, Santos MF, Brandino HE, Martinez R, de Jesus Guirro RR. Red and infrared laser therapy inhibits in vitro growth of major bacterial species that commonly colonize skin ulcers. *Lasers in Medical Science* 2016; **31**: 549-56.

Deaton A. Instruments, randomization, and learning about development. *Journal of Economic Literature* 2010; **48**: 424-55.

Feres M, Haffajee AD, Goncalves C, *et al*. Systemic doxycycline administration in the treatment of periodontal infection (II) effect of antibiotic resistance of subgingival species. *J Clin Periodontal* 1999; **26**(12):784-792.

George S, Hamblin MR, Kishen A. Uptake pathways of anionic and cationic photosensitizers into bacteria. *PhotochemPhotobiol Sci.* 2009; **8**: 788-95.

Giannopoulou C, Cappuyns I, Cancela J, Cionca N, Mombelli A. Effect of photodynamic therapy, diode laser, and deep scaling on cytokine and acute phase protein levels in gingival crevicular fluid of residual periodontal pockets. *J Periodontol* 2012; **83**: 1018-27.

Haffajee AD, Dibart S, Kent Jr RL, Socransky SS. Factors associated with different response to periodontal therapy. *J. Clin. Periodontol.* 1995; **22**: 628-636.

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 Photodyn Ther*. 2019;**26**:29-35.

Jia L, Jia J, Xie M, Zhang X, Li T, Shi L, Shi H, Zhang X. Clinical attachment level gain of lasers in scaling and root planing of chronic periodontitis: a network meta-analysis of randomized controlled clinical trials. *Lasers in Medical Science* 2020; **35**: 473-85.

Khattri S, Nagraj SK, Arora A, Eachempati P, Kusum CK, Bhat KG, Johnson TM, and Lodi G. Adjunctive systemic antimicrobials for the non-surgical treatment of periodontitis. *Cochrane Database of Systematic Reviews* 2020; **11**: CD012568.

Konopka KR, Goslinski TO. Photodynamic therapy in dentistry. *Journal of Dental Research* 2007; 86: 694-707.

Löe H, and Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;**21**: 533-551.

Lindhe J, Westfelt E, Nyman S, Socransky SS, Heijl L, Bratthall G. Healing following surgical non-surgical treatment of periodontal disease: A clinical study. *J ClinPeriodontol.* 1982; **9**: 115-28.

Lesaffre E, Philstrom B, Needleman I, Worthington H. The design and analysis of split-mouth studies: what statisticians and clinicians should know. *Statistics in Medicine*. 2009;**10**: 3470-82.

Listagarten MA. Periodontal probing: what does it mean? *J. Clin Periodontol* 1980; 7(3): 165-76

Merchat M, Bertolini G, Giacomini P, Villanueva A. and Jori G. Meso substituted cationic porphyrins as efficient photo sensitizers of gram positive and gram-negative bacteria. *J Photochem Potobiol* 1996; **32**: 152-157.

Marshall MV, Rasmussen JC, Tan IC, Aldrich MB, Adams KE, Wang X, Fife CE, Maus EA, Smith LA, Sevick-Muraca EM. Near-infrared fluorescence imaging in humans with indocyanine green: a review and update. *Open Surgical Oncology Journal* 2010; **2**: 12.

Mills MP, Rosen PS, Chambrone L, Greenwell H, Kao RT, Klokkevold PR, McAllister BS, Reynolds MA, Romanos GE, Wang HL. American Academy of Periodontology best evidence consensus statement on the efficacy of laser therapy used alone or as an adjunct to nonsurgical and surgical treatment of periodontitis and peri-implant diseases. *J Periodontol* 2019; **89**(7): 737-742.

Newbrun E. Indices to measure gingival bleeding. *J Periodontol.* 1996; **67**(6): 555-61.

Orlandi M, Muñoz AE, Marletta D, Petrie A, Suvan J, and D'Aiuto F. Impact of the treatment of periodontitis on systemic health and quality of life: A systematic review. *J Clin Periodontol* Jun 49: Suppl 2022; **24**: 314-327.

Page RC, and Kornman KS. The pathogenesis of human periodontitis: an introduction. *Periodontol 2000* 1997; **14**: 9-11.

Pretzl B, Sälzer S, Ehmke B, Schlagenhauf U, Dannewitz B, Dommisch H, Eickholz P, and Jockel-Schneider Y. Administration of systemic antibiotics during non-surgical periodontal therapy-a consensus report. *Clin Oral Investig*. 2019; **23**(7): 3073-3085.

Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, *et al.* Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018; **45 Suppl 20**: 162-70.

Qadri T, Javed F, Johannsen G, Gustafsson A. Role of diode lasers (800–980 nm) as adjuncts to scaling and root planing in the treatment of chronic periodontitis: a systematic review. *Photomed Laser Surg*. 2015; **33**: 568-75.

Ranjitha M, Srirangarajan S, Ravi RJ, Srikumar P. Rudresh V. Utility of procalcitonin as an early diagnostic marker of bacteremia in individuals with periodontitis Stage II and III. *J Periodontol*. 2021; **92**: 968-74.

Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol 2000*. 2002;**28**:12-55.

Soukos NS, and Goodson JM. . Photodynamic therapy in the control of oral biofilms. *Periodontol 2000* 2011; **55**: 143-66.

Srirangarajan S, Ranjith M, Ravi RJ, Srikumar Prabhu, Oradiyath D. Are diagnostic inflammatory biomarkers suitable to predict periodontal disease activity after nonsurgical periodontal therapy? *J Int Acad Periodontol* 2022; **24**: 265-72.

Slots J, Mashimo P, Levine MJ, Genco RJ. Periodontal therapy in humans. I. Microbiological and clinical effects of a single course of periodontal scaling and root planing, and of adjunctive tetracycline therapy. *J Periodontol*. 1979; **50**: 495-509.

Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clinical infectious diseases* 2004; **39**: 206-17.

Selvadurai K, Varadhan KB, Venkatesh PM. Effects of non-surgical periodontal therapy on procalcitonin levels of gingival crevicular fluid and serum in subjects with different periodontal conditions. *J Int Acad Periodontol* 2019; **21**: 111- 117.

Xue D, Tang L, Bai Y, Ding Q, Wang P, Zhao, Y. Clinical efficacy of photodynamic therapy adjunctive to scaling and root planing in the treatment of chronic periodontitis: A systematic review and meta-analysis. *Photodiagnosis Photodyn Ther.* 2017; **18**: 119-27.

Yang MC, Marks RG, Magnusson I, Clouser B, Clark WB. Reproducibility of an electronic probe in relative attachment level measurements. *J Clin Periodontol* 1992; **19**(8): 541-8.