PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SRP IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CLINICAL AND MICROBIOLOGICAL STUDY

Dissertation

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BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirements for the degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTICS

By

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Under the guidance of

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

(Faculty of Babu Banarasi Das University)

BATCH: 2014-2017

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "***PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SRP IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CLINICAL AND MICROBIOLOGICAL STUDY***" is a bonafide and genuine research work carried out by me under the guidance of ***Dr. VIVEK GOVILA***, Principal, Professor and Head, Department of Periodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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"Education is not preparation for life; Education is life itself"

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they have been providing throughout my educational career and therefore, I dedicate this work to them as a token of my respect.

Last, but not the least, I thank the almighty and ever loving "GOD".

Dr. Rajiv Kumar Singh

Enrolment Number: 1140328005

DEDICATED

TO

MY FAMILY

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LIST OF ILLUSTRATIONS

ABBREVIATIONS

A.a	A. actinomycetemcomitans
BOP	Bleeding on probing
CP	Chronic periodontitis
CAL	Clinical attachment level
CLSM	Confocal laser scanning micrograph
EMD	Enamel matrix derivative
LLLT	Low level laser therapy
LB	Luriabertani medium
MB	Methylene blue
NSPD	Nonsurgical periodontal therapy
PCR	Polymerase Chain Reaction
PDT	Photodynamic therapy
PS	Photosensitizer
PI	Plaque index
PPD	Probing pocket depths
SRP	Scaling and root planning
TBO	Toluidine blue-O

Abstract

Eradication or suppression of pathogens is the major goal in periodontal therapy. Due to the increase in antibiotic resistance, the need for new disinfection therapies has risen in recent times. One such method, photodynamic therapy (PDT) has demonstrated anti-infective potential. The aim of this study was to investigate the clinical and microbiological adjunctive outcome of photodynamic therapy with scaling and root planing (SRP), compared to SRP alone in the treatment of chronic periodontitis. In this split-mouth design study, 30 chronic periodontitis patients (mean age, 37.5 years) with probing pocket depth (PPD) >4mm, clinical attachment level (CAL) > 2mm were included. After clinical and radiographic assessment, two quadrants were randomly selected in each subject who would receive either PDT along with SRP (PDT GROUP) or SRP alone (SRP GROUP). Plaque Index (PI), PPD and CAL were recorded and subgingival plaque samples were collected for microbiological analysis. Clinical parameters and plaque sample analysis was done at baseline and 6 months post treatment. After 6 months, mean PI decreased from 1.60 to 1.23 in SRP group and from 1.60 to 1.19 in the PDT group. The mean PPD decreased from 2.67 mm to 2.12 mm in SRP group and from 2.61 mm to 2.04 mm in the PDT group while mean CAL decreased from 3.40 mm to 2.86 mm in SRP group and from 3.31 mm to 2.67 mm in the PDT group. Microbiologically, higher reductions of *p. gingivalis* were observed in PDT group. This study showed that adjunctive photodynamic treatment may enhance clinical and microbiological outcome in patients with chronic periodontitis.

Keywords: Chronic periodontitis, Photodynamic therapy

Introduction

Chronic periodontitis (CP) is a chronic inflammatory response to the accumulation of microbial plaque and calculus on the root surface of the tooth; this condition leads to breakdown of the surrounding periodontal tissues.[1] The aim of periodontal treatment is to restore the biological compatibility of periodontally diseased root surfaces for subsequent attachment of periodontal tissues to the treated root surface.[2] The gold standard for the non-surgical treatment of periodontal disease is scaling and root planing (SRP). In SRP, the removal of supragingival and subgingival biofilms along with the diseased root surface is facilitated using hand instruments and ultrasonic scalers. However, complete removal of the bacterial biofilm and their endotoxins in deeper areas of the pockets and furcation sites is often difficult to achieve with both methods.[3]

To deal with this clinical issue, a number of adjunctive therapies have been developed over the years. In general these treatment modalities include systemic administered antibiotics and local delivery of antiseptics or antibiotics. Although these have been considered to eliminate residual periodontal pathogens after scaling and root planing, studies have shown that an organized biofilm exhibits several resistance and mechanisms that protect the periodontal pathogens and limit the action of the antibiotics.[4]

Recently, photodynamic therapy (PDT) has been introduced in periodontal therapy in an attempt to improve the effectiveness and efficiency of root surface debridement and bacterial elimination.

PDT can be defined as the eradication of target cells by reactive oxygen particles produced by means of a photosensitizing compound and light of an appropriate wavelength (630-830 nm).[5]

This action was first observed in 1900 by Raab, who realized that a protozoon could be killed in the presence of acridine dye, excited by a visible light.[6] Although PDT is more widely known for its application in the treatment of neoplasms, it shows great potential in the treatment of periodontitis, because many species were reported to be killed in vitro by this approach.[7]

PDT involves three components: light, a photosensitizer, and tissue oxygen. Laser is the preferred source of light for photodynamic therapy because it emits coherent, monochromatic, intense and unidirectional light[8] while methylene blue and toluidine blue O are very effective photosensitizing agents for the inactivation of both gram-positive and gram-negative bacteria.[9] Upon irradiation, the photosensitizer undergoes a transition from a low energy ground state to a higher energy triplet state, which can then react with biomolecules to produce free radicals (type I reaction), or with molecular oxygen to produce highly reactive singlet oxygen (type II reaction),leading to cell death.[10] **[PLATE I]**

The present study aims to comparatively evaluate the efficacy of photodynamic therapy as an adjunct to SRP in the treatment of chronic periodontitis patients with the help of clinical and microbiological parameters.

Aim & Objectives

The **aim** of the present study was:

- To clinically and microbiologically evaluate the effectiveness of photodynamic therapy as an adjunct to scaling and root planing in patients with chronic periodontitis.

The **objectives** of the present study were:

1) To conduct a randomized in vivo clinical trial to assess the efficacy of one time application of photoactivated dye, toluidine blue-O with diode laser in treating patients with chronic periodontitis.
2) The primary endpoint of the clinical trial was to evaluate and compare a significant gain in clinical attachment level (CAL) at baseline and six months post treatment.
3) The secondary endpoints were to evaluate and compare the reduction in probing pocket depths (PPD) and plaque scores (plaque index) at baseline and six months post treatment.

Review of Literature

Photodynamic therapy is a unique and interesting therapeutic approach towards periodontal therapy. The numerous in vitro and in vivo studies have clearly demonstrated its effective and efficient bactericidal effect and is extensively studied in periodontics due to its technical simplicity and its ability to eradicate bacteria, the causative agents behind occurrence of periodontal diseases.

Goharkhay K, Moritz A, Smith PW, Schoop U, Kluger W, Jakolitsch S (1999)[11] determined incision characteristics and soft-tissue damage resulting from standardized incisions using a wide range of laser modes and parameters of a diode laser at 810 nm. The remarkable cutting ability and the tolerable damage zone clearly showed that the diode laser is a very effective and, because of its excellent coagulation ability, useful alternative in soft-tissue surgery of the oral cavity.

Simunovic Z, Ivankovich AD, Depolo A (2000)[12] evaluated animal and clinical studies to assess the efficacy of low level laser therapy (LLLT) on wound healing in rabbits and humans. Results showed that wound healing was significantly accelerated (25%-35%) in patients treated with LLLT. Pain relief and functional recovery of patients treated with LLLT were significantly improved compared to untreated patients.

Schwarz F, Sculean A, Georg T, Reich E (2000)[13] evaluated and compared the effectiveness of an Er:YAG laser to that of scaling and root planing for non-surgical periodontal treatment. Clinical assessments of plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), gingival recession (GR) and clinical attachment level (CAL) were made prior to and at 3 and 6 months after treatment. The results showed that there was reduction in the BOP score and the CAL improvement was significantly higher in the laser group than in the SRP group. Additionally, both groups

showed a significant increase of cocci and non-motile rods and a decrease in the amount of motile rods and spirochetes.

Schwarz F, Sculean A, Georg T, Becker J (2003)[14] in a randomized controlled trial concluded that Er:YAG laser and enamel matrix derivative (EMD) does not seem to improve the clinical outcome of the therapy additionally compared with SRP+EDTA+EMD. They concluded that both therapies led to short-term improvements of the investigated clinical parameters, and the combination of Er:YAG and EMD does not seem to improve the clinical outcome of the therapy additionally compared to SRP+EDTA+EMD.

Sculean A, Schwarz F, Windisch P, Keglevich T, Gerharz D, Becker J (2005)[15] performed flap surgeries of intrabony defects using Er:YAG laser and demonstrated that healing was predominantly characterized by formation of the long junctional epithelium along the instrumented root surface and connective tissue attachment was accompanied by bone regeneration and no thermal damage was observed.

Yukna RA, Carr RL, Evans GH (2007)[16] performed non-surgical treatment of chronic periodontitis with a free running pulsed Nd:YAG laser to remove the pocket epithelium. All laser treated specimens showed new cementum and new connective tissue attachment in and occasionally coronal to the notch whereas specimens treated with just scaling and root planing had a long junctional epithelium with no evidence of new attachment or regeneration.

Derdilopoulou FV, Nonhoff J, Neumann K, Keilbassa AM (2007)[17] conducted a study to evaluate and compare the microbiological effects of hand instrumentation, Er:YAG

laser, sonic and ultrasonic scalers in patients with chronic periodontitis. Three months post-operatively, the amounts of Pg, Pi, Tf, and Td were significantly reduced in all groups. Laser and sonic instrumentation failed to reduce A.a. Six months after therapy, significant differences were still detected for Pg (L- and U-group), for Pi and Tf (S-group), and for Td (L-, S- and U-group).

Almeida JM, Theodoro LH, Bosco AF, Nagata MJ, Oshiiwa M, Garcia VG (2007)[18] in a study to assess radiographically the effect of photodynamic therapy (PDT) as an adjunctive treatment to scaling and root planing (SRP) on induced periodontitis in dexamethasone-induced immunosuppressed rats. The results showed that there was statistically significant less bone loss in the animals treated with PDT in all experimental periods compared to those submitted to SRP. Hence they concluded that PDT was an effective adjunctive treatment to SRP on induced periodontitis in dexamethasone-induced immunosuppressed rats.

Oliveira RR, Schwartz-Filho HO, Novaes AB Jr, Taba M Jr (2007)[19] conducted a clinical trial for assessing the efficacy of PDT for nonsurgical treatment of periodontitis in comparison with SRP. At three months following the therapy, both treatment showed comparable outcomes in terms of bleeding on probing and pocket depth, gains in clinical attachment level, and thus suggesting a potential clinical benefit of PDT.

Braun A, Dehn C, Krause F, Jepsen S (2008)[20] performed a split mouth clinical trial to determine the efficacy of PDT as an adjunct to SRP in the treatment of chronic periodontitis. All teeth received periodontal treatment comprising scaling and root planing. Using a split-mouth design, two quadrants (test group) were additionally treated

with PDT. Sulcus fluid flow rate (SFFR), bleeding on probing (BOP), relative attachment level (RAL) and probing depths (PDs) were assessed at baseline, 1 week and 3 months after treatment. The results showed that clinical outcomes of conventional subgingival debridement can be improved by adjunctive PDT.

Christodoulides N et al (2008)[21] conducted a study to evaluate the clinical and microbiologic effects of the adjunctive use of PDT to non-surgical periodontal treatment. They showed that the addition of a single episode of PDT to SRP failed to result in an improvement in terms of PD reduction and CAL gain, but it resulted in a significantly greater reduction in bleeding scores compared to SRP alone.

Polansky R, Haas M, Heschl A, Wimmer G (2009)[22] conducted a randomized-controlled clinical trial to evaluate photodynamic therapy for its bactericidal potential and clinical effect in the treatment of periodontitis. Baseline clinical values of gingival index, bleeding on probing, probing pocket depths and clinical attachment levels were recorded and re-evaluated 90 days later. Pathogen screening for P. gingivalis, Tannerella forsythia and Treponema denticola were conducted at baseline as well as 10, 42 and 90 days after treatment. The results showed that application of a single cycle of PDT was effective as an adjunct to ultrasonic periodontal treatment. There were extra reductions in pocket depths and bleeding on probing. With regard to eradicating bacteria, however, there are no additional effects as compared with conventional treatment alone.

Al-Zahrani MS, Bamshmous SO, Alhassani AA, Al-Sherbini MM (2009)[23] conducted a clinical trial to assess the effect of PDT on periodontal status and blood sugar of diabetic patients with chronic periodontitis. The plaque and bleeding scores, probing

depth, clinical attachment level, and glycosylated hemoglobin (HbA1c) level were recorded at baseline and 3 months after periodontal treatment. The results of the study showed significant differences in periodontal parameters and but not on the glucose levels at the baseline and after 3 months.

Fontana CR et al (2009)[24] investigated the ability of PDT to reduce the number of bacteria in biofilms by comparing the photodynamic effects of methylene blue (MB) on human dental plaque microorganisms in the planktonic phase and in biofilms. In their study, multispecies microbial biofilms developed from the same plaque samples were exposed to methylene blue and the same light conditions as their planktonic counterparts. After PDT, survival fractions were calculated by counting the number of colony-forming units and the results showed that PDT killed approximately 63% of bacteria present in suspension. By contrast, in biofilms, PDT had much less of an effect on the viability of bacteria (32% maximal killing).

Oliveira RR et al (2009)[25] conducted a clinical trial to investigate cytokine levels in the gingival crevicular fluid (GCF) of patients with aggressive periodontitis, after treatment with PDT or SRP. The results of the study showed that non-surgical periodontal treatment with PDT or SRP led to statistically significant reductions in TNF-alpha level 30 days following treatment. There were similar levels of TNF-alpha and RANKL at the different time points in both groups, with no statistically significant differences.

Seguier S, Souza SL, Sverzut AC, Simioni AR, Primo FL (2010)[26] conducted a clinical trial to evaluate the effects of PDT on the inflammatory infiltrate and on the collagen network organization in chronic periodontitis. In the study, each patient was

depth, clinical attachment level, and glycosylated hemoglobin (HbA1c) level were recorded at baseline and 3 months after periodontal treatment. The results of the study showed significant differences in periodontal parameters and but not on the glucose levels at the baseline and after 3 months.

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treated with PDT using phthalocyanine derivatives as photosensitizers and the contralateral tooth was taken as control. The findings of the study demonstrated that PDT presents an impact on gingival inflammatory phenomenon during chronic periodontitis and leads to a specific decrease of antigen-presenting cells populations (macrophages and Langerhans cells).

Qadri T, Miranda L, Tuner J, Gustafsson A. (2010)[27] performed a split-mouth, double-blind controlled clinical trial to study the effects of irradiation with low-level lasers as an adjunctive treatment of inflamed gingival tissue. The test side was treated with two low-level lasers having wavelengths of 635 and 830 nm. The GCF samples were analysed for elastase activity, interleukin-1beta (IL-1beta) and metalloproteinase-8 (MMP-8). The clinical variables i.e. probing pocket depth, plaque and gingival indices were reduced more on the laser side than on the placebo side after three months. However elastase activity, IL-1beta concentration and the microbiological analyses showed no significant differences between the laser and placebo sides.

Sigusch BW, Engelbrecht M, Volpel A, Holletschke A, Pfister W (2010)[28] conducted a study to clinically and microbiologically evaluate the effect of PDT as a full-mouth procedure in Fusobacterium nucleatum infected patients with periodontitis. In patients with chronic periodontitis who received PDT treatment, significant reductions in BOP, and mean PD and CAL were observed during the observation period with respect to controls. Also 12 weeks after treatment, the F. nucleatum DNA concentration was found to be significantly reduced compared to the baseline level.

De Souza RC, Lima FF, Alegretti CE, Scabar LF, Martins RB (2011)[29] studied the use of PDT with methylene blue dye 0.01% in the treatment of chronic periodontitis. The results proved to be an effective adjuvant in periodontal therapy with manual debridement, accelerating the process of tissue regeneration, decontamination, reduction of pain, and significant improvement of parameters of periodontal health in patients with chronic periodontitis.

Al-Zahrani MS, Austah ON (2011)[30] conducted a split mouth clinical trial in order to compare the efficacy of PDT as an adjunct with SRP for treatment of chronic periodontitis in smokers. Plaque index (PI), bleeding on probing (BOP), probing depth (PD), recession and clinical attachment level (CAL) were recorded at baseline and 3 months after the periodontal treatment in two groups i.e. PDT group and SRP group. The results showed that PDT have an additional benefit to scaling and root planing when treating smokers affected with periodontitis.

Ge et al (2011)[31] evaluated the clinical effects of PDT with SRP in the treatment of chronic periodontitis. PDT was performed at sites with a probing depth (PD) ≥5 mm using PDT. Periodontal values of bleeding on probing (BOP), pocket depth (PD) and clinical attachment level (CAL) were examined at baseline, 6 weeks and 12 weeks after treatment. The results showed that, compared to the baseline, sites with PD ≥5 mm showed significant reductions of PD, CAL, and BOP at 6 and 12 week after treatment.

Mettraux G, Husler J (2011)[32] conducted a clinical trial for evaluating the efficacy of PDT as an adjunct to scaling and root planing for treatment of chronic periodontitis. The transgingival application of PDT showed clinical and bacteriological effects which are

comparable to those reported in the literature with the subgingival method. Thus they concluded that the transgingival method is convenient, harmless and easy to perform compared to subgingival method.

Walter Dukic, Ivona Bago, Andrej Aurer, Marija Rogulji (2012)[33] conducted a randomized clinical trial to evaluate the effect of 980-nm diode laser as an adjunct to SRP in the treatment of chronic periodontitis. The study indicated that, compared to SRP alone, multiple adjunctive applications of a 980-nm diode laser with SRP showed PD improvements in moderate periodontal pockets (4 to 6 mm).

Dilsiz A, Canakci V, Aydin T (2012)[34] conducted a split mouth clinical trial to assess and compare the efficacy of potassium-titanyl-phosphate (KTP) laser and PDT for treatment of chronic periodontitis. In the study, teeth in each quadrant were randomly treated by SRP alone (group A), PDT followed by SRP (group B), or KTP laser followed by SRP (group C). All treatments yielded significant improvements in terms of BOP and PD decrease and CAL gain compared to baseline values. Group C showed a greater reduction in PD compared to the other groups. In addition, group C showed a greater CAL gain compared to the other groups.

Theodoro LH et al (2012)[35] through a split mouth clinical trial done with the purpose of assessing the long term clinical and microbiological effects of PDT in conjunction with non-surgical periodontal therapy for treatment of chronic periodontitis showed that PDT decreased some key pathogens but had significant effects on the clinical parameters recorded at baseline and 6 months after treatment.

Berakdar M, Callaway A, Eddin MF, Ross A, Willershausen B (2012)[36] conducted a clinical trial to evaluate and compare the efficacy of PDT in conjunction with SRP in chronic periodontitis patients. The following clinical parameters were assessed at baseline, one, three and six months after the therapy: bleeding on probing (BOP), plaque index (PI), probing depth (PD) and clinical attachment loss. The results demonstrated that SRP in combination with PDT seems to be effective and is therefore suitable as an adjuvant therapy to the mechanical conditioning of the periodontal pockets in patients with chronic periodontal diseases.

Javed F, Qadri T, Ahmed BH, Al-Hezaimi K, Corbet FE (2013)[37] performed a clinical study to assess whether or not photodynamic therapy with adjunctive scaling and root planing is effective in the treatment of periodontitis under immunocompromised conditions. They found that SRP with PDT to be ineffective in treating chronic periodontitis in type II diabetes mellitus patients. All experimental studies reported significantly less bone loss in periodontal defects treated with SRP and PDT than those treated with SRP alone. However the efficacy of SRP and PDT in the treatment of periodontal disease under immunocompromised conditions remained unclear.

Cappuyns I, Cionca N, Wick P, Giannopoulou C, Mombelli (2012)[38] compared the efficacy of PDT with diode laser plus SRP in management of residual pockets in chronic periodontitis through a split mouth clinical trial. Residual pockets >4 mm were debrided with an ultrasonic device and then subjected to either PDT or SRP. Pocket probing depth (PPD), bleeding on probing (BOP) and gingival recession were monitored over 6 months. Counts of three microorganisms were determined by direct hybridization with RNA

probes. Both treatments resulted in a significant clinical improvement and also suppressed Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola.

Alwaeli HA, Al-Khateeb SN, Al-Sadi A (2013)[39] in a split mouth clinical trial done with the purpose of assessing the long term clinical efficacy of PDT associated with SRP in treatment of chronic periodontitis showed that PDT as an adjunct to scaling and root planning can be a novel approach for treatment of periodontitis.

Queiroz AC et al (2013)[40] through a split mouth clinical trial conducted with the aim of assessing the efficacy of PDT as an adjunct to SRP in smokers with chronic periodontitis. Plaque index, bleeding on probing, probing depth, clinical attachment level and gingival recession were recorded, and gingival crevicular fluid was collected for assay of IL-1β and matrix metalloproteinase (MMP)-8 levels. The results obtained after three months showed that there were no statistically significant differences in intragroup comparisons. The adjunctive effect of PDT did not warrant improvements on clinical parameters in smokers. However, it resulted in a suppression of IL-1β and MMP-8 when compared with SRP alone.

Luchesi VH et al (2013)[41] conducted a clinical trial done to evaluate the effect of PDT as an adjunct to SRP in the treatment of Class II furcation. Clinical, microbiological and cytokine pattern evaluation was performed at baseline, three months and six months and clinical attachment level was defined as the primary outcome variable. The results showed that clinical parameters improved after both therapies with no differences between groups at any time point. However, IFN-γ, IL-6, IL-8 and IL-1β levels were lower in the PDT group.

Petelin M, Perkic K, Seme K, Gaspirc B (2014)[42] conducted a study to compare the effect of subgingival ultrasonic scaling followed by repeated (three times) antimicrobial photodynamic therapy, ultrasonic scaling alone (US) and scaling and root planing with hand instruments (SRP) for initial periodontal treatment. The results showed that additional application of PDT to US failed to result in further improvement in terms of PPD reduction and CAL gain. However, it resulted in a higher reduction of BOP at 3 and 12 months comparing to US alone or SRP. Additionally, PDT resulted in a greater reduction of Aggregatibacter actinomycetemcomitans and Tannerella forsythia in moderate pockets (4-6 mm) compared to mechanical debridement alone.

Moreira AL et al (2014)[43] performed a split mouth clinical trial done to assess the efficacy of multi-step PDT as an adjunct to SRP in the treatment of aggressive periodontitis.The results showed that the test group (PDT group) presented a decrease in PD and a clinical attachment gain significantly higher than the control group (SRP group) at 90 days. The test group also demonstrated significantly less periodontal pathogens of red and orange complexes and a lower interleukin-1β/interleukin-10 ratio than the control group.

Arweiler NB et al (2014)[44] evaluated and compared the outcomes following nonsurgical periodontal therapy and additional use of either PDT or amoxicillin and metronidazole in patients with Aggressive Periodontitis. In their study, at least three sites with pocket depth (PD) ≥6 mm were treated with SRP and either systemic administration of metronidazole for 7 days or with two episodes of PDT and clinical parameters were evaluated at baseline and at 6 months. The results showed that while both treatments resulted in statistically significant clinical improvements, metronidazole showed

statistically significantly higher PD reduction and lower number of pockets ≥6 mm compared to PDT.

Chitsazi MT et al (2014)[45] performed a clinical trial done to assess the efficacy of PDT in treatment of aggressive periodontitis. In a split-mouth design study, the teeth of one quadrant was treated with PDT having a wavelength of 670-690 nm and a power of 75 Mw (PDT group). The control group consisted of selected teeth of the contralateral quadrant treated with SRP only. Treatment groups showed an improvement in all the clinical parameters and a significant reduction in the counts of A. actinomycetecommitans at 90 days compared to baseline.

Queiroz AC et al (2014)[46] in their study to assess the microbiological effects of PDT as an adjunct to SRP for non-surgical periodontal therapy in smokers with chronic periodontitis found neither PDT associated with SRP nor SRP alone decreased the microbial count in smokers.

Pourabbas R et al (2014)[47] conducted a study to compare the effectiveness of adjunctive photodynamic therapy (PDT) in the treatment of aggressive periodontitis. In a split-mouth design study, PDT was performed with a diode laser beam with a wavelength of 670-690 nm and a power of 75 mW (test group) while the control group consisted of selected teeth of the contralateral quadrant treated with SRP only. Treatment groups showed an improvement in all the clinical parameters and a significant reduction in the counts of *A. actinomycetecommitans* at 90 days compared to baseline.

Betsy J, Prasanth CS, Baiju KV, Prasanthila J, Subhash N (2014)[48] evaluated the effect of PDT as an adjunct to SRP in the treatment of chronic periodontitis. In their

study, untreated chronic periodontitis were randomly assigned to receive SRP with PDT (test group) or SRP alone (control group) and clinical parameters and halitosis were recorded at baseline and six months after treatment. The results showed statistically significant reduction in probing pocket depth and gain in the clinical attachment levels at 3 months and 6 months as compared to the control group.

Chitsazi MT, Shirmohammadi A, Shirmohammadi M, Kashefimehr A, Ghasemi V (2015)[49] evaluated and compared the clinical and microbiological effectiveness of adjunctive photodynamic therapy in the treatment of periodontitis. In their study twenty-four subjects diagnosed with moderate to severe chronic periodontitis underwent scaling and root planing. One tooth in each quadrant (probing depth >4 mm) was selected for combined PDT and SRP (PDT group) with the contralateral tooth (SRP group), as a control site (SRP-treated site). Clinical measurements showed significant decreases after one and three months at both sites, without inter-group differences, except for bleeding on probing after one and three months.

Birang R, Shahaboui M, Kiani S, Shadmehr E, Naghsh N (2015)[50] conducted a study to evaluate the impact of adjunctive laser therapy (LT) and photodynamic therapy (PDT) on patients with chronic periodontitis. The clinical indices namely CAL, PPD, papilla bleeding index and microbiological samples were evaluated at baseline and 3-months. The obtained data suggested that adjunctive LT and PDT had significant short-term benefits in the treatment of chronic periodontitis.

Teymouri F, Farhad SZ , Golestaneh H (2016)[51] studied the impact of photodynamic therapy on the level of gingival crevicular fluid (GCF), inflammatory mediators and

periodontal clinical status. Significant reduction was observed over time in the level of IL-1β, IL-17, clinical attachment loss and pocket depth in the three treatment groups at the baseline, up to 2 weeks and 2-6 weeks. In addition, photodynamic therapy significantly decreased the average bleeding on probing over time.

Annaji S, Sarkar I, Rajan P (2016)[52] evaluated the efficacy of PDT as an adjunct to conventional scaling in the treatment of patients with aggressive periodontitis. The clinical parameters included PI, BOP, PPD, CAL and were recorded at the baseline and three months and bacterial sampling and culture for Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis and Prevotella intermedia were done. The results showed statistically significant reduction in clinical & microbial parameters in the group treated with PDT and scaling.

Materials & Methods

Materials & Methods

The following study was conducted in the Department of Periodontology, BBD College of Dental Sciences, Lucknow, Uttar Pradesh in collaboration with Department of Microbiology, Central Drug Research Institute (CDRI), Lucknow. The aim of the study was to comparatively evaluate the efficacy of photodynamic therapy as an adjunct to SRP in the treatment of chronic periodontitis patients with the help of clinical and microbiological aids.

STUDY DESIGN

A total of 30 subjects reporting to the OPD of Department of Periodontics aged between 25-50 years were selected for the study.

The study confirmed to the ethical guidelines of the Helsinki Declaration and was evaluated and approved by the Institutional Ethical Committee. A written informed consent was obtained from all subjects participating in the study.

The study protocol included a customized proforma for systematic recording of the observations and information. A detailed medical and dental case history record for clinical examination and periodontal charting were recorded.

The patients were selected based upon the following inclusion and exclusion criteria.

INCLUSION CRITERIA

1. Patients within the age group of 25-50 years.

2. Patients having chronic periodontitis defined as having minimum of 20 remaining teeth, with periodontal disease as evidenced by at least 4 tooth sites with probing pocket depth (PPD) >4mm, clinical attachment level (CAL) > 2mm and radiographic

evidence of bone loss >2mm from the cementoenamel junction (CEJ).[53]

3. Patients who were cooperative and committed to maintain oral hygiene.

4. Patients with no contraindication to periodontal therapy.

EXCLUSION CRITERIA

1. Patients suffering from chronic systemic illness like diabetes and active infections and taking medication for the same.

2. Patients suffering from aggressive periodontitis, periodontal abscess, necrotizing ulcerative gingivitis or periodontitis.

3. Patients who have undergone any periodontal treatment or antibiotics within the preceding 3 months.[54]

STUDY GROUPS:

The study protocol was explained to all patients and those who fulfilled the criteria were enrolled in the study. After clinical and radiographic assessment, two quadrants were randomly selected in each subject which would receive either scaling and root planing along with photodynamic therapy (PDT GROUP) or scaling and root planing alone (SRP GROUP).**[PLATE IV]**

PDT GROUP: 30 Quadrants that were treated with PDT along with SRP.

SRP GROUP: 30 Quadrants that were treated with SRP alone.

Criteria for selecting PDT Group are as follows:

1. Subjects with ≥20 natural teeth present with chronic periodontitis.
2. Periodontal disease was evidenced by at least 4 tooth sites with probing pocket depth (PPD) >4mm, clinical attachment level (CAL) >2mm and radiographic evidence of bone loss >2mm from the cementoenamel junction (CEJ).

Criteria for selecting SRP Group are as follows:

1. Subjects with ≥20 natural teeth present with chronic periodontitis.
2. Periodontal disease was evidenced by at least 4 tooth sites with probing pocket depth (PPD) >4mm, clinical attachment level (CAL) >2mm and radiographic evidence of bone loss >2mm from the cementoenamel junction (CEJ).

ARMAMENTARIUM

A) PERIODONTAL EXAMINATION AND SCALING AND ROOT PLANING

- Mouth mirror
- University of North Carolina Probe (UNC-15)
- Explorer
- Tweezer
- Gloves
- Face mask
- Headcap
- Scaler (Satelec P5®)
- Gracey Curettes (Hu-friedy™) **[PLATE II]**

B) PHOTODYNAMIC THERAPY

- Diode Laser {Fotosan™ (wavelength 620-640nm), CMS Dental }

- Photosensitizer {Fotosan™ (Toluidine Blue O, conc. 0.1 mg/ml),CMS Dental)
- Applicator Tip
- Insulin Syringe
- Disposable Syringe
- Saline
- Protective Eyewear **[PLATE III]**

C) MICROBIOLOGICAL ANALYSIS

- Gracey Curettes (Hu-friedy™)
- Transport media (LURIA BERTANI)
- Incubater
- Selective media for P.gingivalis

CLINICAL PARAMETERS

Clinical parameters were recorded and plaque samples were collected. These plaque samples were then subjected to microbiological analysis.

The following clinical parameters were recorded:

1. Plaque Index (Sillness and Loe, 1964)[55]
2. Probing Pocket Depth (PPD)
3. Clinical Attachment Level (CAL)

Probing pocket depth (PPD) and clinical attachment level (CAL) were measured at four surfaces of all teeth (mid-buccal, mid-lingual, mesial and distal inter-proximal sites) to

the nearest millimeter, using UNC-15 probe, having markings from 1 to 15 and colour coded at an interval of 5, 10 and 15.

SPECIMEN COLLECTION

After removal of supra-gingival plaque and calculus, the area was dried and isolated with cotton rolls and saliva evacuators and sub-gingival plaque samples were collected using sterile curettes (Hu-Friedy, USA.) from each selected site (i.e. deepest pocket in each quadrant) and was immediately transferred to the transport media. Sample was transported as early as possible to the microbiological laboratory for culturing.

SCALING AND ROOT PLANING PROCEDURE

Full mouth Scaling and root planing (SRP) was performed with the help of ultrasonic scaler {Satelec P5® SATELEC (India) PVT. Ltd Gandhinagar, India} and gracey curettes (Hu-friedy, USA).

PROCEDURE FOR APPLICATION OF PDT

After full mouth scaling and root planing, PDT was performed with a diode laser with power settings at 630nm wavelength, 2mW output and continuous mode irradiated with toluidine blue-O (1mg/ml) solution as a photosensitizer in one of the quadrant (PDT GROUP) while the other selected quadrant acted as a placebo (SRP GROUP). The photosensitizer was applied to the bottom of the periodontal pocket with the help of an insulin syringe. After 3 minutes of action the photosensitizer was rinsed with saline and exposed to diode laser for 30 seconds at each site. The procedure was done using standard laser safety protocol including protective eye wear for the operator and the patient. **[PLATE V]**

MICROBIOLOGICAL ANALYSIS

Subgingival plaque was collected by universal curette and transported in vials containing luriabertani (LB) medium to microbiology laboratory. The medium was then incubated for 24 hrs. After this, culture plates were prepared in laboratory using the plaque sample. Also a selective medium were prepared for P.gingivalis.**[PLATE VI]**

Selective medium for P.gingivalis

It was prepared by coloumbia agar which is supplemented with sheep blood, bacitracin, colistin and nalidixic acid. This medium was prepared, stored and dispensed under oxygen-free conditions to prevent formation of oxidized products prior to use. **[PLATE VII]**

Bacterial count

Bacterial count was done by 10 fold dilution and plating method at baseline and 6 months post-operatively.

Confirmation test of P. Gingivalis

Identification of organism was confirmed by real-time PCR (Polymerase Chain Reaction) using specific primers.

Isolation of DNA from plaque samples and bacterial cultures

The *P. gingivalis* culture dilution and plaque samples (100 ul) were used for automated DNA extraction and purification with the MagNA Pure DNA Isolation Kit III. The

protocol included 1 hour of pretreatment with proteinase K (20 mg/ml) at 56°C. After isolation, the DNA was diluted in 100 ul of elution buffer.

To monitor the efficacy of the DNA isolation method, all samples were spiked with a known amount (1,000 CFU) of an *Escherichia coli* culture before DNA isolation.

PCR primers and probes

The 16S rRNA sequences of the genus *Porphyromonas* were selected from the taxonomy database of the Division of microbiology at Central Drug Research Institute, Lucknow.

A sequence alignment by using the multiple-alignment tool in the MagNA program was performed to search for homologous sequences within the 16S rRNA. The sequence of *P. gingivalis* W83 was used to select the primer and TaqMan probe sequences in a region of maximal homology by using Primer Express software. This software generated series of best combinations for the *P. gingivalis* primer and probe set. The combinations were checked for primer-dimer or internal hairpin configurations, melting temperature, and percent G_C values.

The sequence of the forward primer, primer P.g.F, was 5-GCGCTCAACGT TCAGCC-3 (base pairs 612 to 628); the sequence of the reverse primer, primer P.g.R, was 5-CACGAATTCCGCCTGC-3 (base pairs 664 to 679) and the sequence of the Taqman probe, probe P.g.P, was 5-CACTGAACTCAAGCCC GGCAGTTTCAA-3 (base pairs 634 to 660).

The oligonucleotide probe was labeled with the fluorescent dyes 6-carboxyfluorescein at the 5 end and 6-carboxytetramethylrhodamine (TAMRA) at the 3 end. The *E.coli* primer-

probe combination was labeled with the fluorescent reporter dye VIC at the 5 end and the quencher dye TAMRA at the 3 end.

Optimization, sensitivity and specificity of *P. gingivalis*-specific primer-probe set

PCRs were performed by using a matrix of concentrations of the forward primer, the reverse primer, and the probe to determine the optimal concentration yielding the lowest threshold cycle (*Ct*) values and, hence, the highest amplification efficiencies.

The specificity of the real-time PCR assay was verified with purified genomic DNA from 10 different bacterial strains.

The detection limit of the real-time PCR was assessed by determining the *Ct* values of serial 10-fold dilutions of purified genomic DNA from *P. gingivalis* strain W83. A standard curve prepared with these dilutions was used in every experiment.

Quantitative PCR assay

PCR amplification was performed in a total reaction mixture volume of 25 ul. The reaction mixtures contained 12.5ul TaqMan universal PCR master mixture (PCR buffer, deoxynucleoside triphosphates, AmpliTaq Gold, an internal reference signal [6-carboxy-X-rhodamine] and uracil *N*-glycosylase,), 300 nM each of *P. gingivalis*-specific primer, 100 nM of *P. gingivalis*-specific probe and 5 ul of purified DNA from plaque samples. Five microliters of the DNA extracted from *P. gingivalis* W83 was used to prepare the standard curve and as a positive control while the negative control was 5ul of sterile H_2O. The samples were subjected to an initial amplification cycle of 50°C for 2 min and 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. The data were

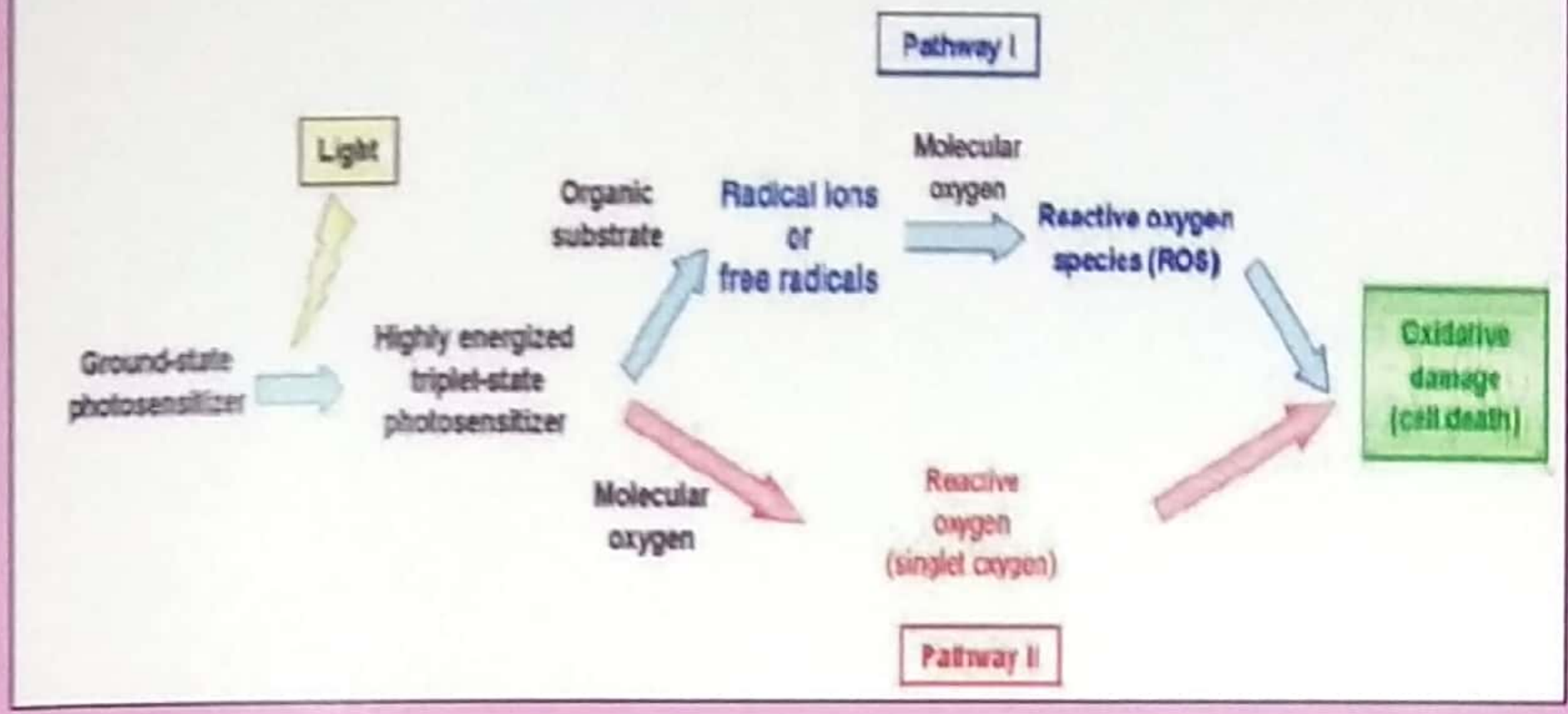
analyzed with ABI 7000 Sequence Detection System software. The degradation of the probe by the DNA polymerase in each elongation step induces an increase in fluorescence that can be monitored during PCR amplification.

The fluorescence signal is normalized by dividing the reporter dye emission (6-carboxyfluorescein) by the emission of the passive reference (6-carboxy-X-rhodamine). The higher the starting copy number of the nucleic acid target is, the sooner a significant increase in fluorescence is observed. The *Ct* parameter is defined as the fractional cycle number at which the fluorescence of the reporter dye generated by cleavage of the probe crosses an arbitrarily defined threshold within the logarithmic phase. Hence, this parameter can be used to compare different amplification reactions. The results for unknown plaque samples were projected on the standard curve generated with *P. gingivalis* strain W83.

Specificity and Sensitivity

The specificity was determined as the number of negative results by the real-time PCR assay divided by the number of negative results by the quantitative culture test. The sensitivity was determined as the number of positive results by the real-time PCR divided by the number of positive results by the quantitative culture test. **[PLATE VIII]**

PHOTODYNAMIC REACTIONS



DIAGRAMMATIC REPRESENTATION OF STEPS INVOLVED IN PDT

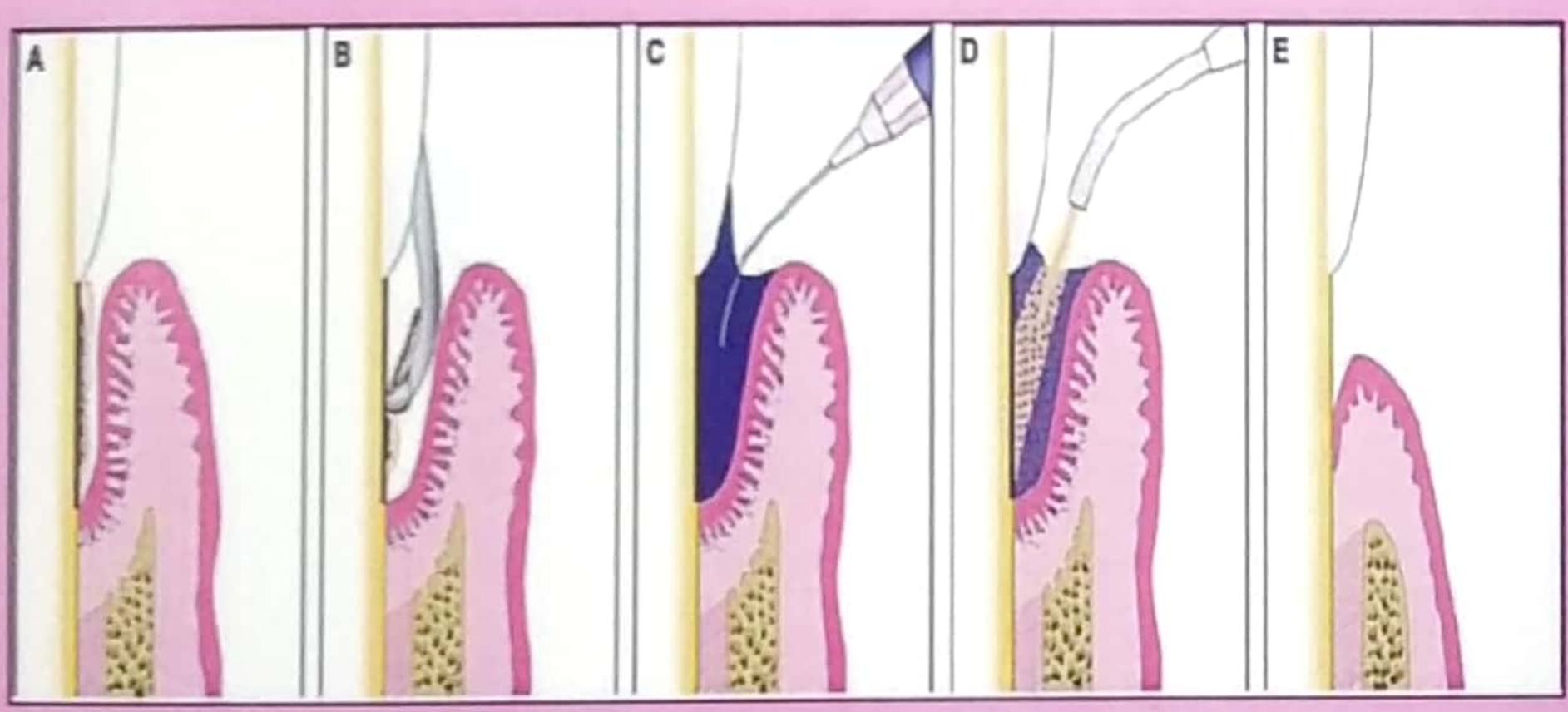
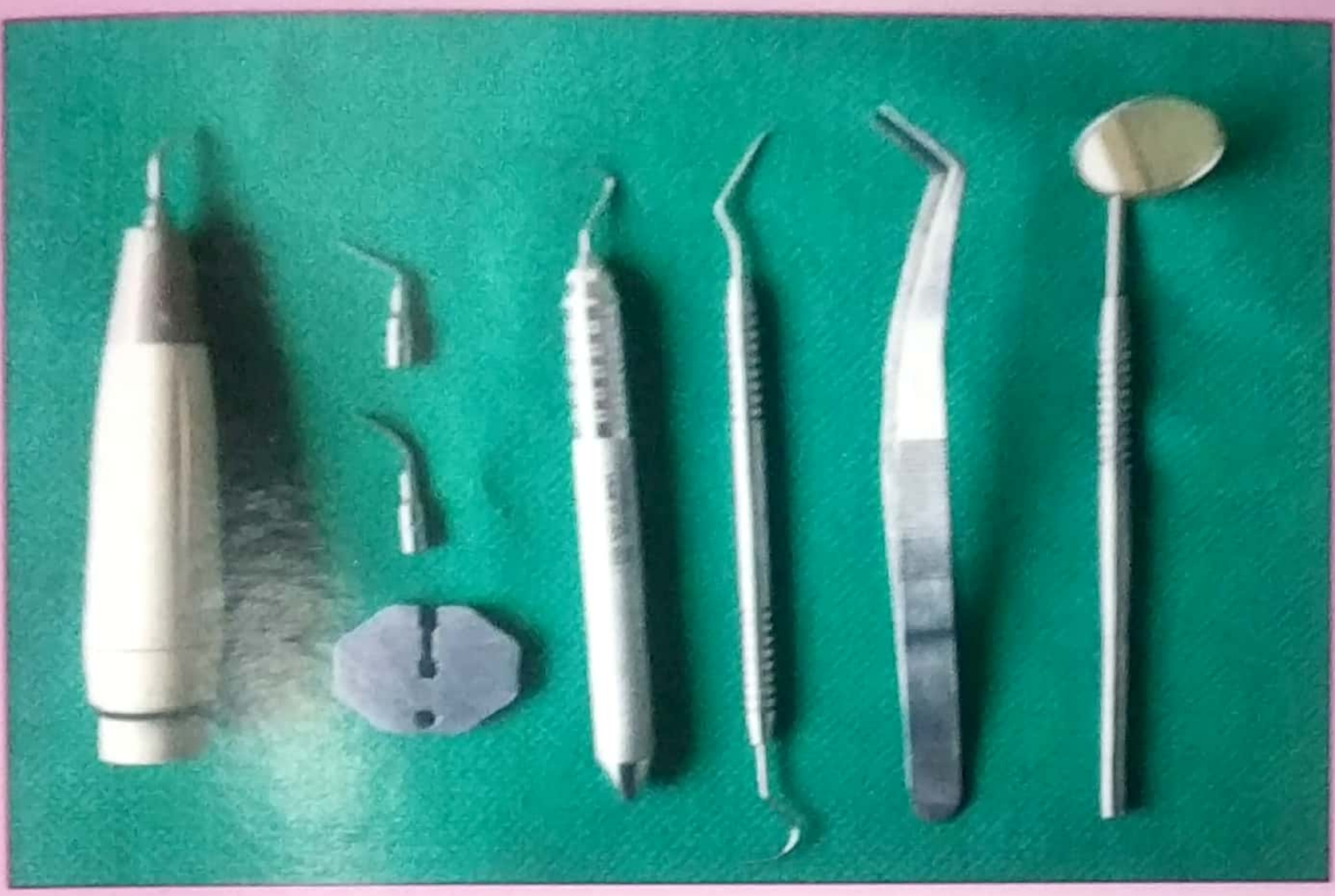


PLATE NO. I

CLINICAL ARMAMENTARIUM USED IN THE STUDY



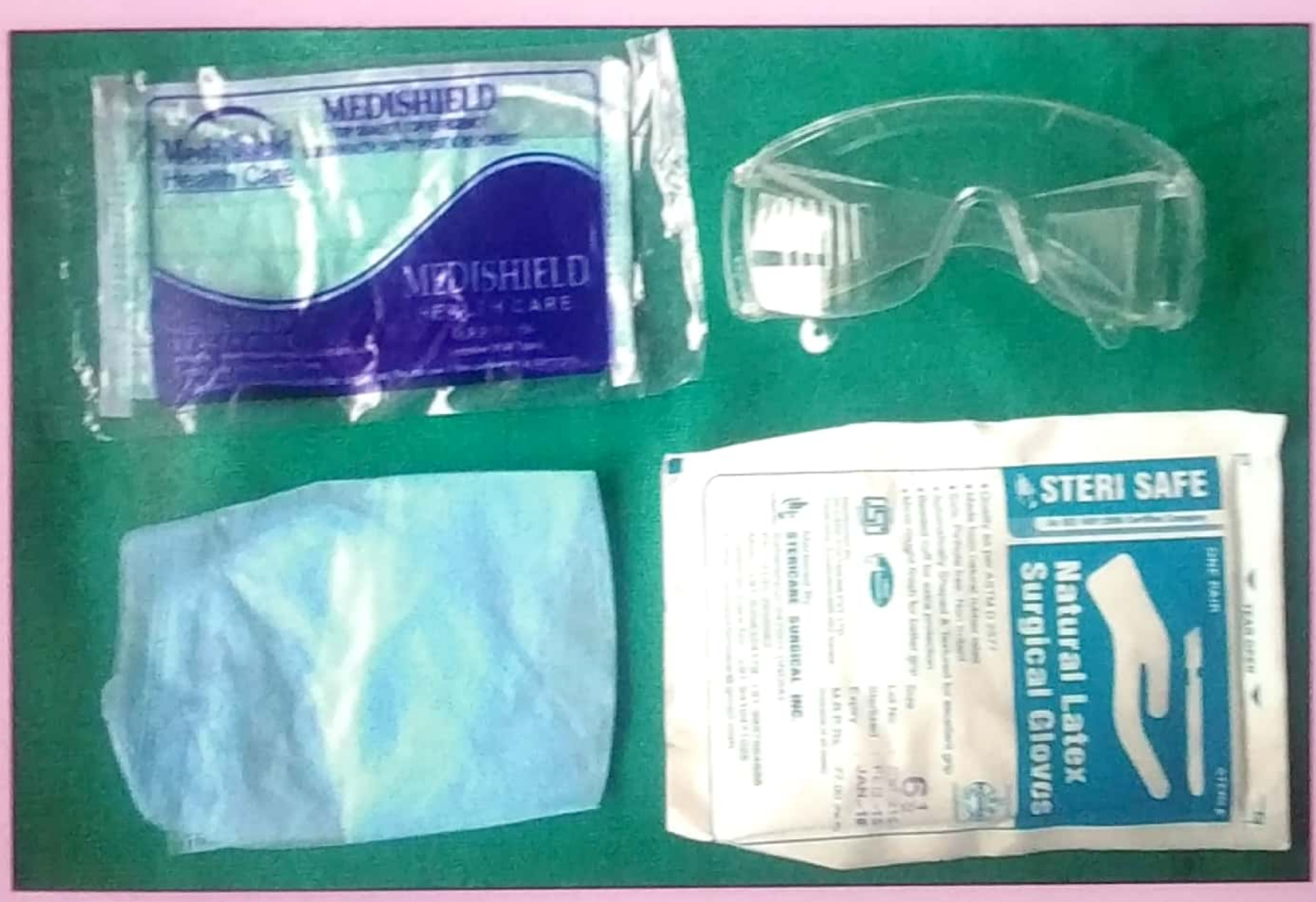
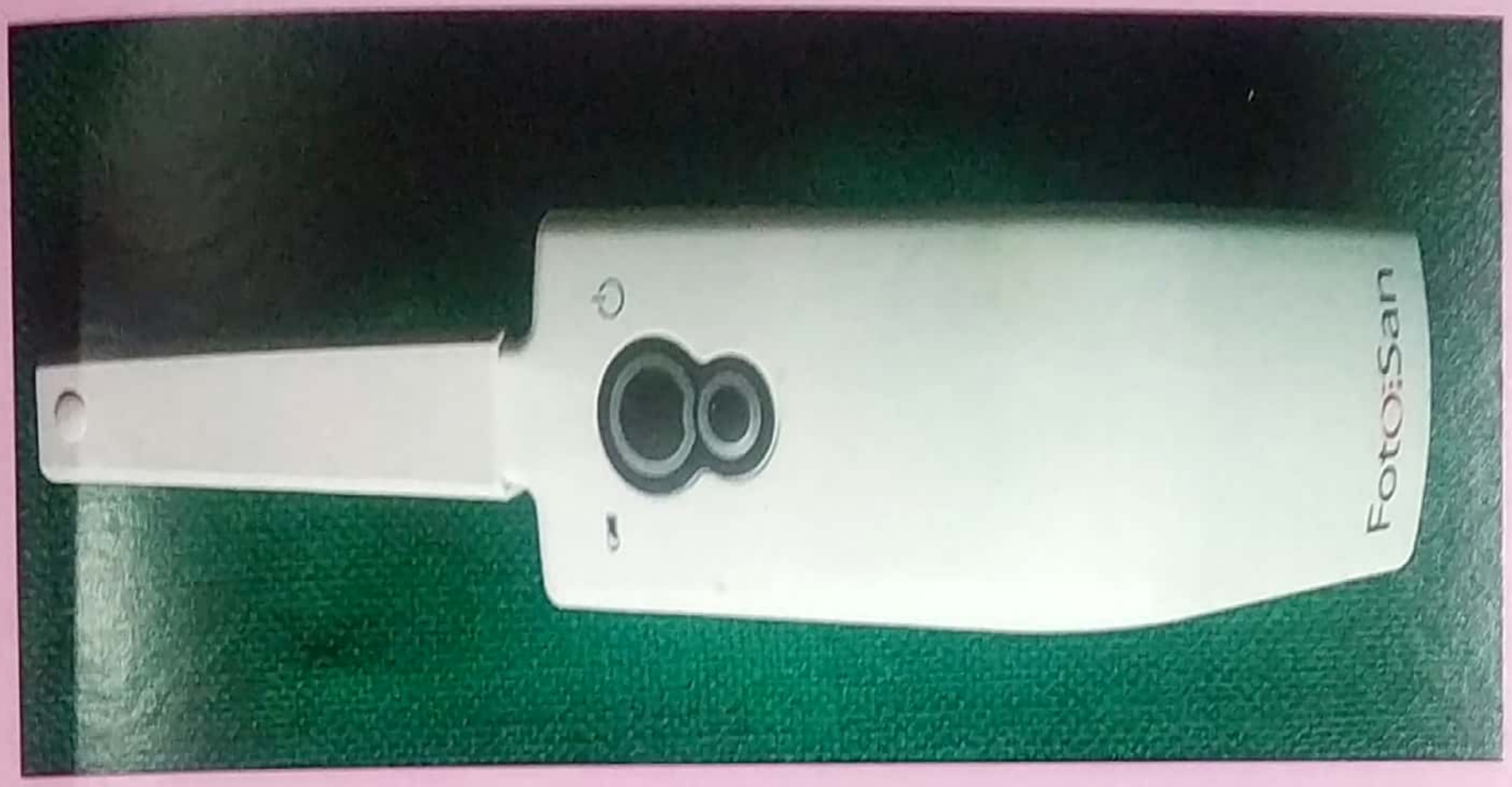


PLATE NO. II

ARMAMENTARIUM USED IN PHOTODYNAMIC THERAPY

DIODE LASER HANDPIECE



PHOTOSENSITIZER (TOLUIDINE BLUE-O)

PLATE NO. III

PREOPERATIVE OPG

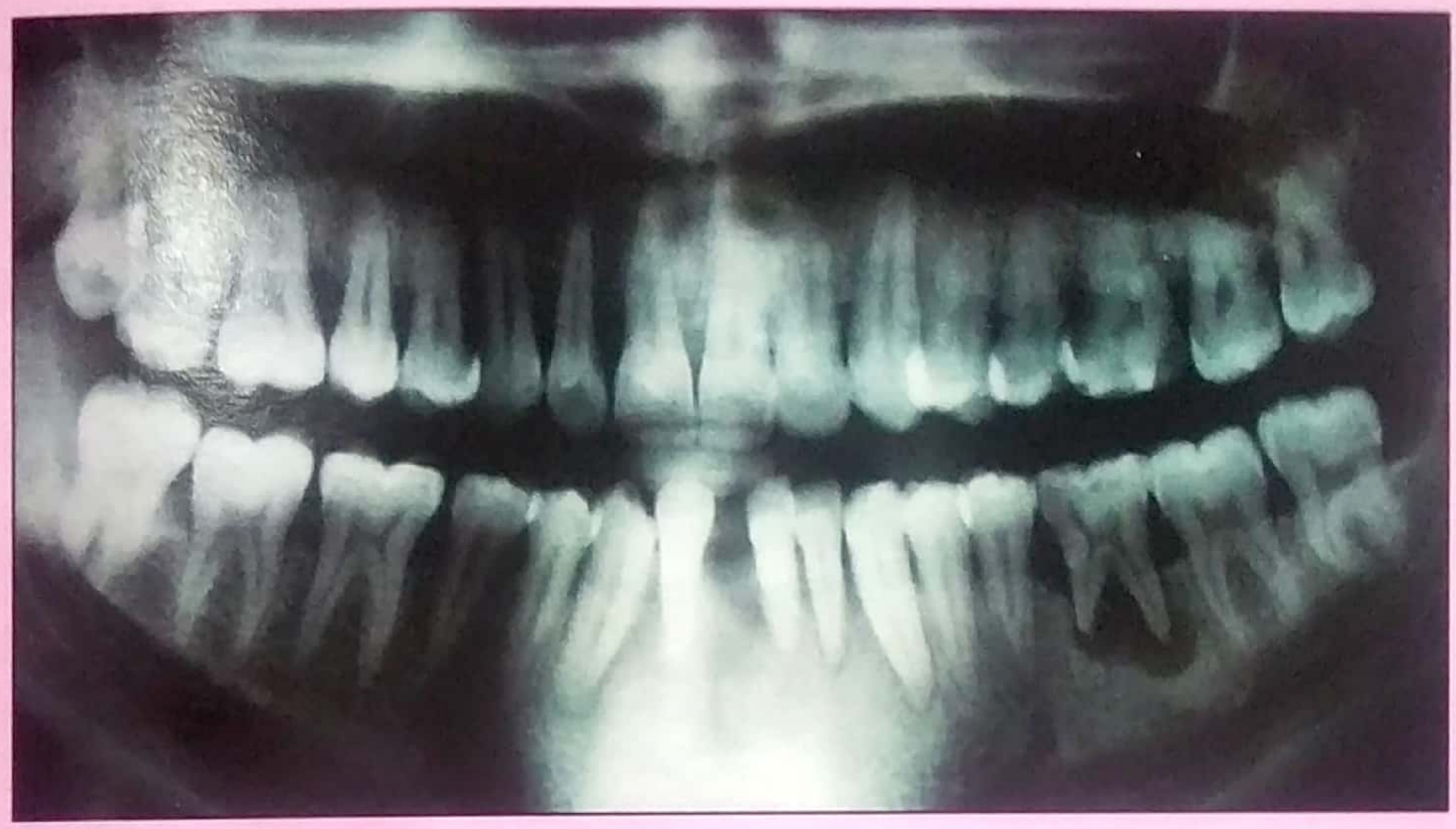
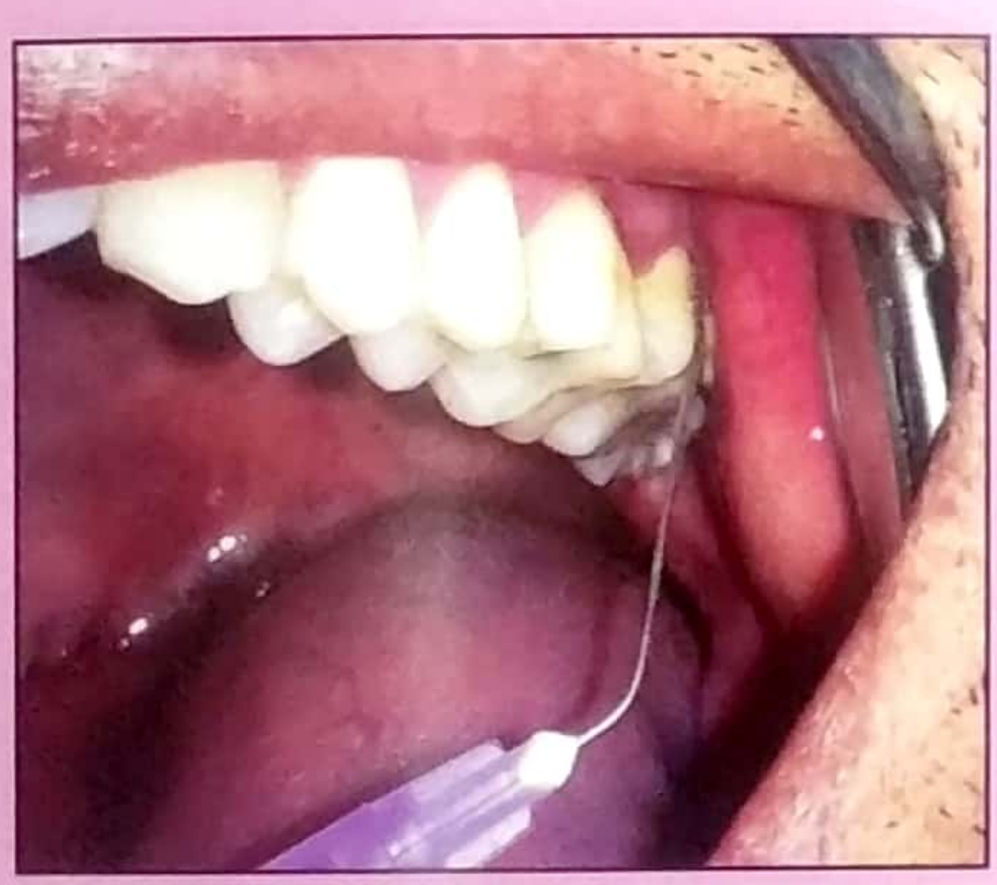


PLATE NO. IV

CLINICAL STEPS INVOLVED IN PHOTODYNAMIC THERAPY



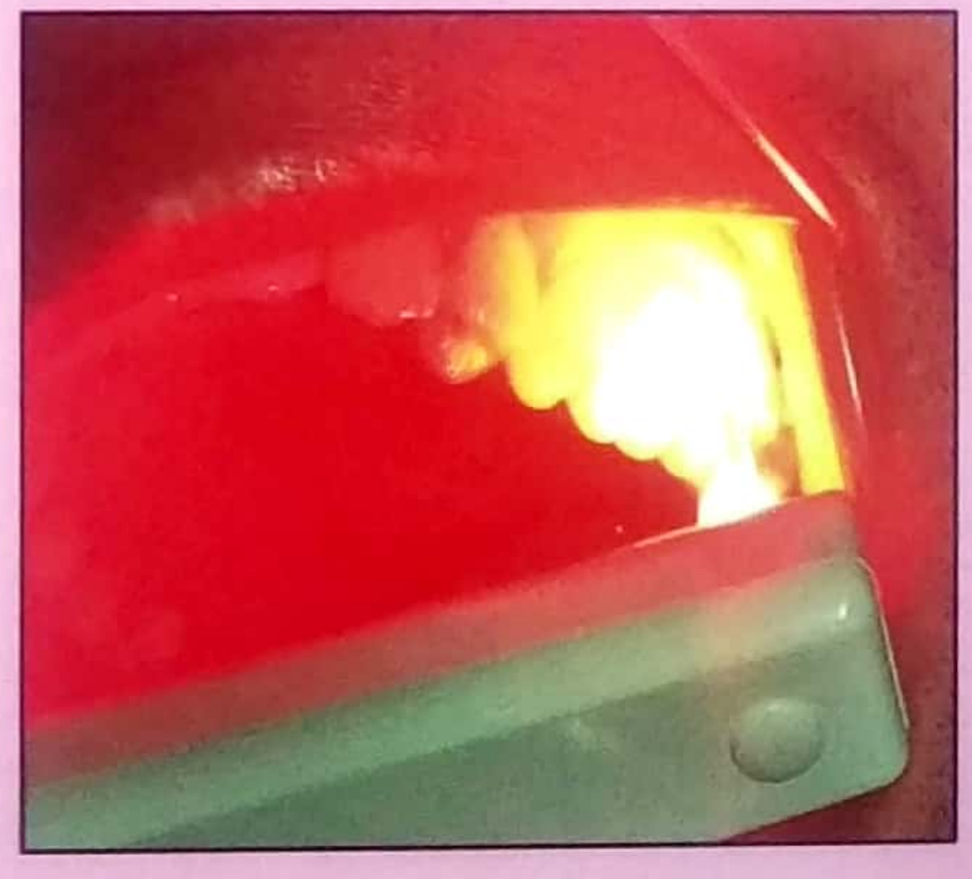
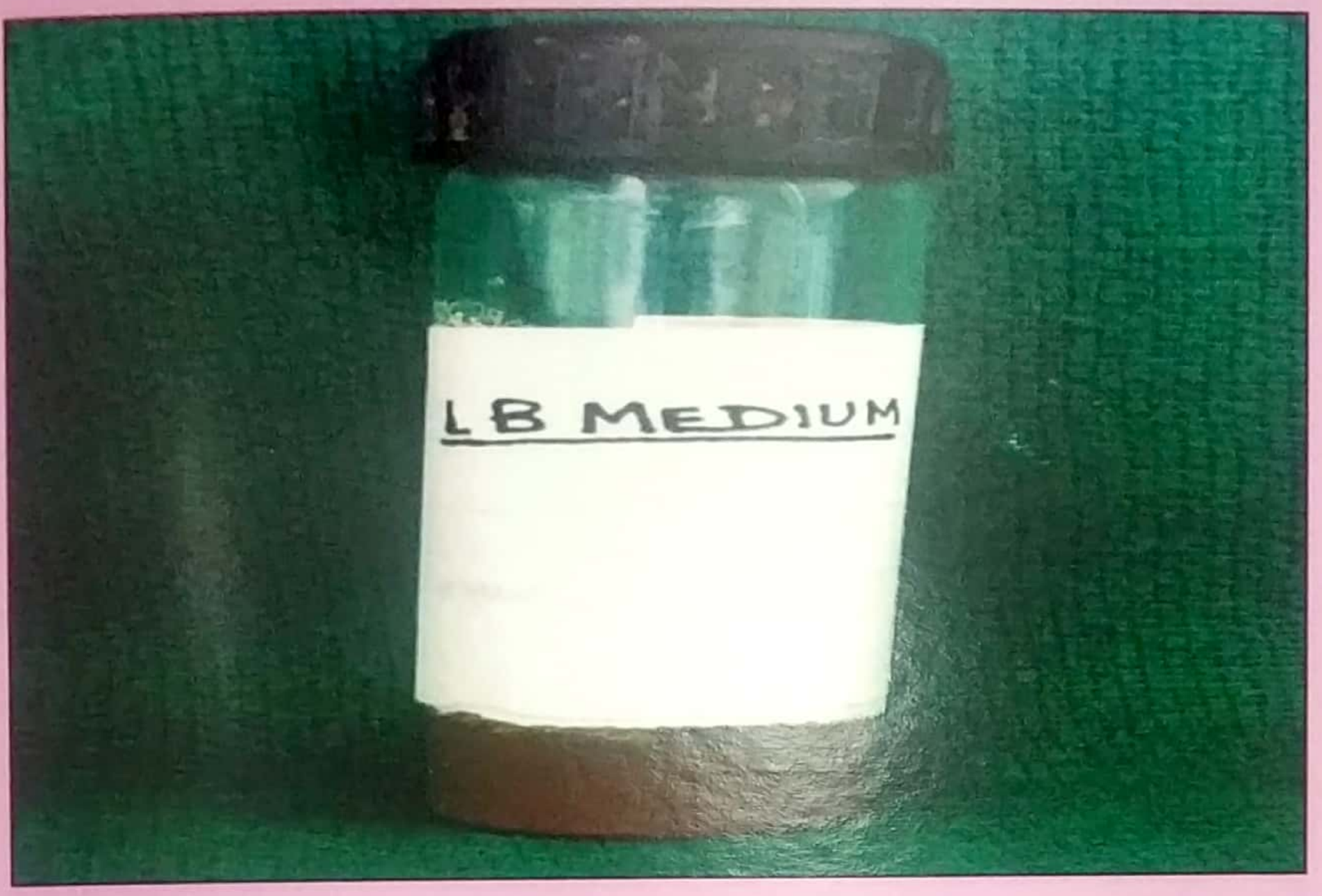


PLATE NO. V

ARMAMENTERIUM FOR TRANSPORTATION OF PLAQUE SAMPLE

i) TRANSPORT (LURIABERTANI) MEDIUM



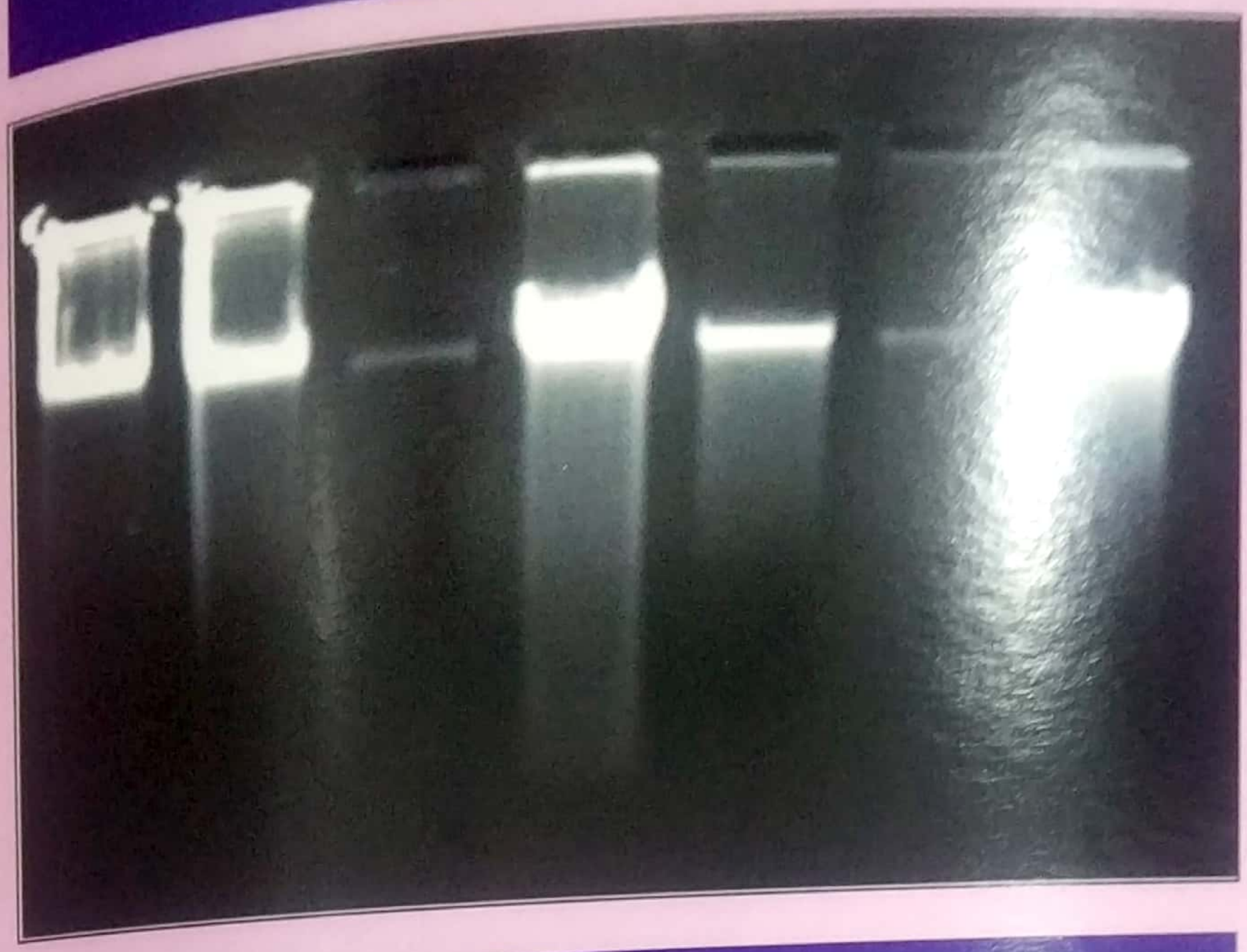
ii) INCUBATOR USED FOR STORAGE OF TRANSPORT MEDIUM

PLATE NO. VI

COLONIES SHOWING GROWTH OF P.GINGIVALIS ON CULTURE PLATE

PLATE NO.VII

GENOMIC DNA ISOLATION OF P.GINGIVALIS (PCR)



DISTINCT BASE PAIRS OF P.GINGIVALIS(PCR)

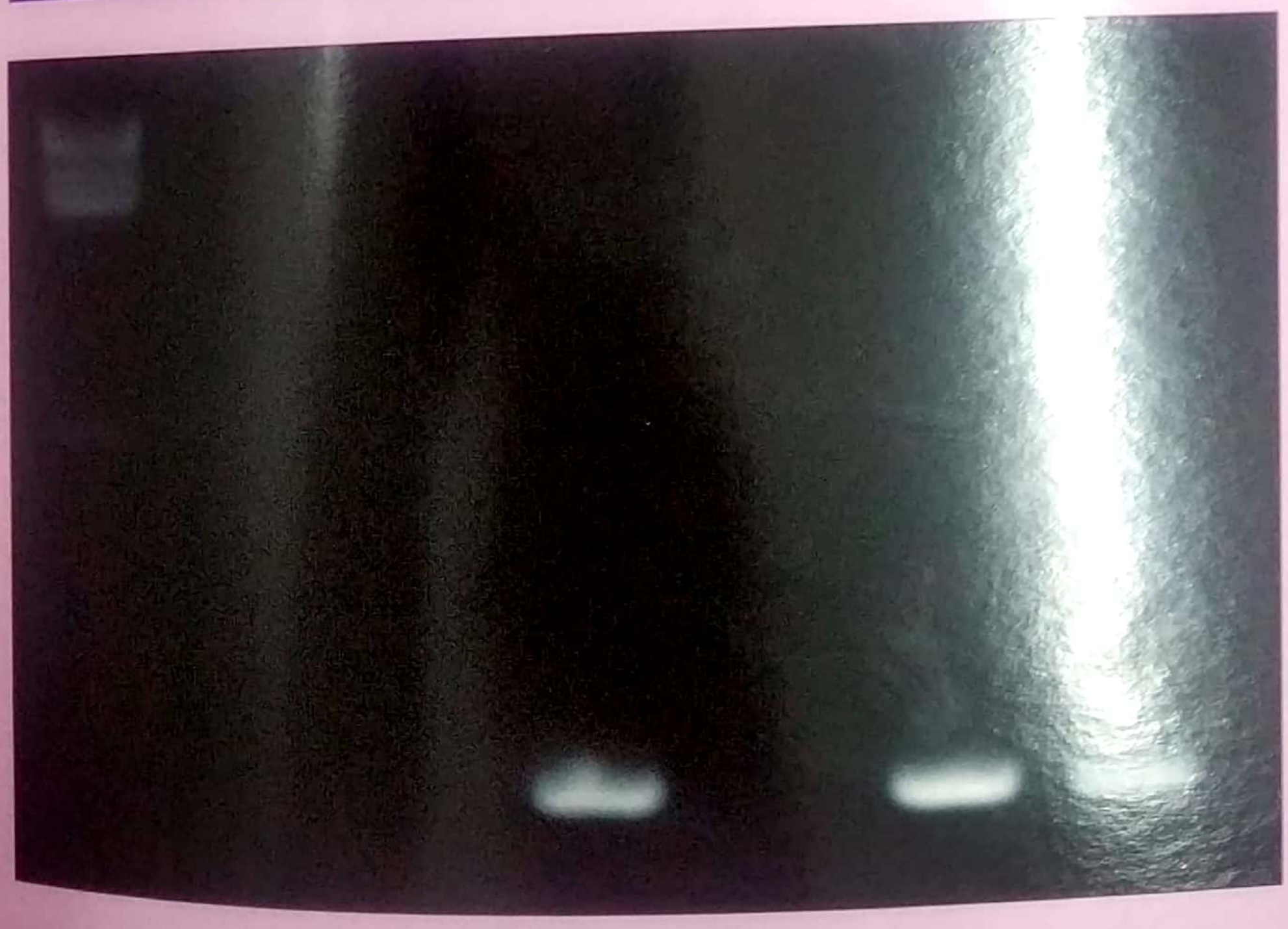


PLATE NO. VIII

Observations & Results

The present clinical and microbiological clinical trial evaluates efficacy of photodynamic therapy as an adjunct to scaling and root planing in the treatment of chronic periodontitis. Total 30 chronic periodontitis patients, age between 25-50 years, either sex were recruited and two quadrants were randomly selected in each patient which would receive either scaling and root planing along with photodynamic therapy (PDT GROUP) or scaling and root planing alone (SRP GROUP) (Table 1 and Fig. 1).

The outcome measures of the study were

I. Clinical

A) Plaque Index (PI)

B) Probing Depth (PD)

C) Clinical Attachment Level (CAL)

II. Microbiological

P. Gingivalis count

All the clinical and microbiological parameters were assessed at pre treatment (baseline) and 6 month post treatment (6 month).

The objective of the study was to compare the outcome measures between the two groups.

Table 1: Allocation of group and distribution of treatment sites

Treatment	Group name	No of Quadrants (n=60) (%)
Scaling and root planing	SRP	30 (50.0)
Scaling and root planing + Photodynamic therapy	PDT	30 (50.0)

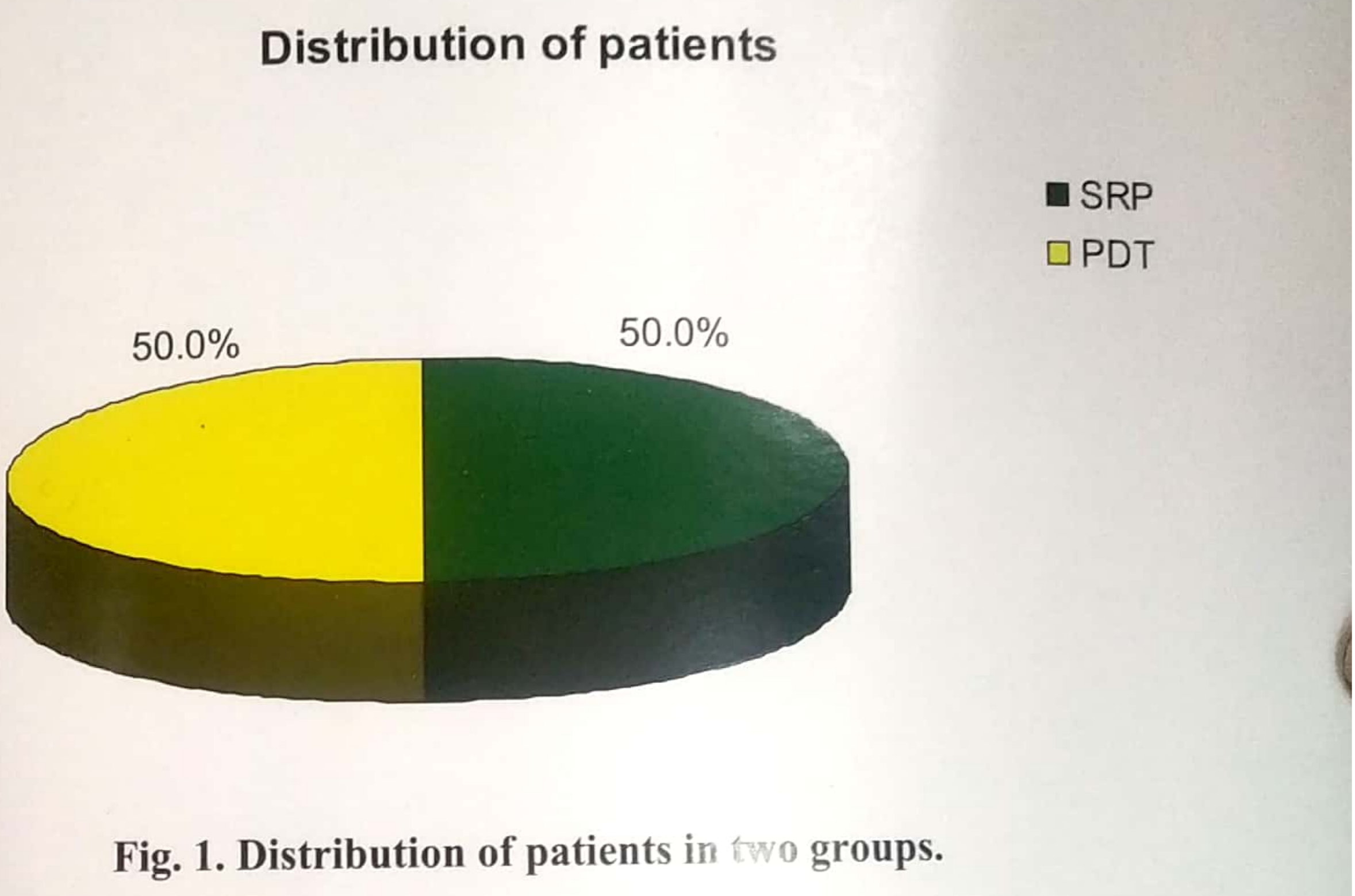


Fig. 1. Distribution of patients in two groups.

Outcome measures

I. Plaque index

The pre (baseline) and post treatment (6 month) PI of two groups (SRP and PDT) is summarised is summarised in Table 2. In both groups, the mean PI decrease comparatively after the treatment and the decrease was evident slightly higher in PDT than SRP.

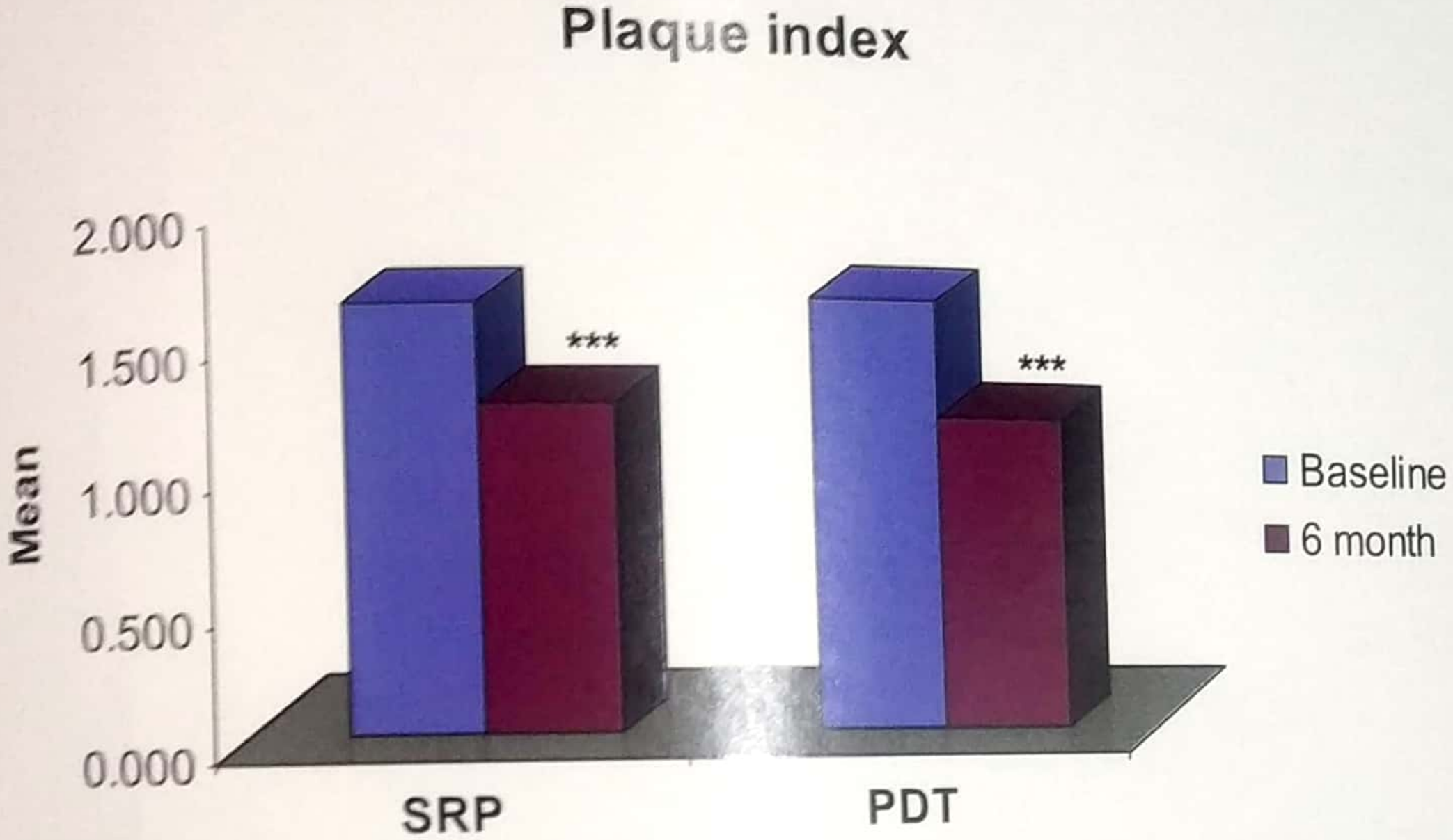
For each group, comparing the mean difference in PI between the periods (baseline vs. 6 month), Tukey test showed significant decrease in PI at 6 month as compared to baseline in both SRP (1.602 ± 0.026 vs. 1.227 ± 0.028, diff=0.376, p<0.001) and PDT (1.625 ± 0.025 vs. 1.183 ± 0.023, diff=0.441, p<0.001) groups (Table 2 and Fig. 2).

Similarly, for each period, comparing the mean difference in PI between the groups (SRP vs. PDT), Tukey test showed similar PI between the groups at both baseline (1.602 ± 0.026 vs. 1.625 ± 0.025, diff=0.022, p=0.929) and 6 month (1.227 ± 0.028 vs. 1.183 ± 0.023, diff=0.043, p=0.634) i.e. did not differ significantly (Table 2 and Fig. 3).

However, at final evaluation (i.e. the mean change from baseline to 6 month), the decrease in PI of PDT group (27.2%) was found to be 3.7% higher (effect size) as compared to SRP group (23.4%).

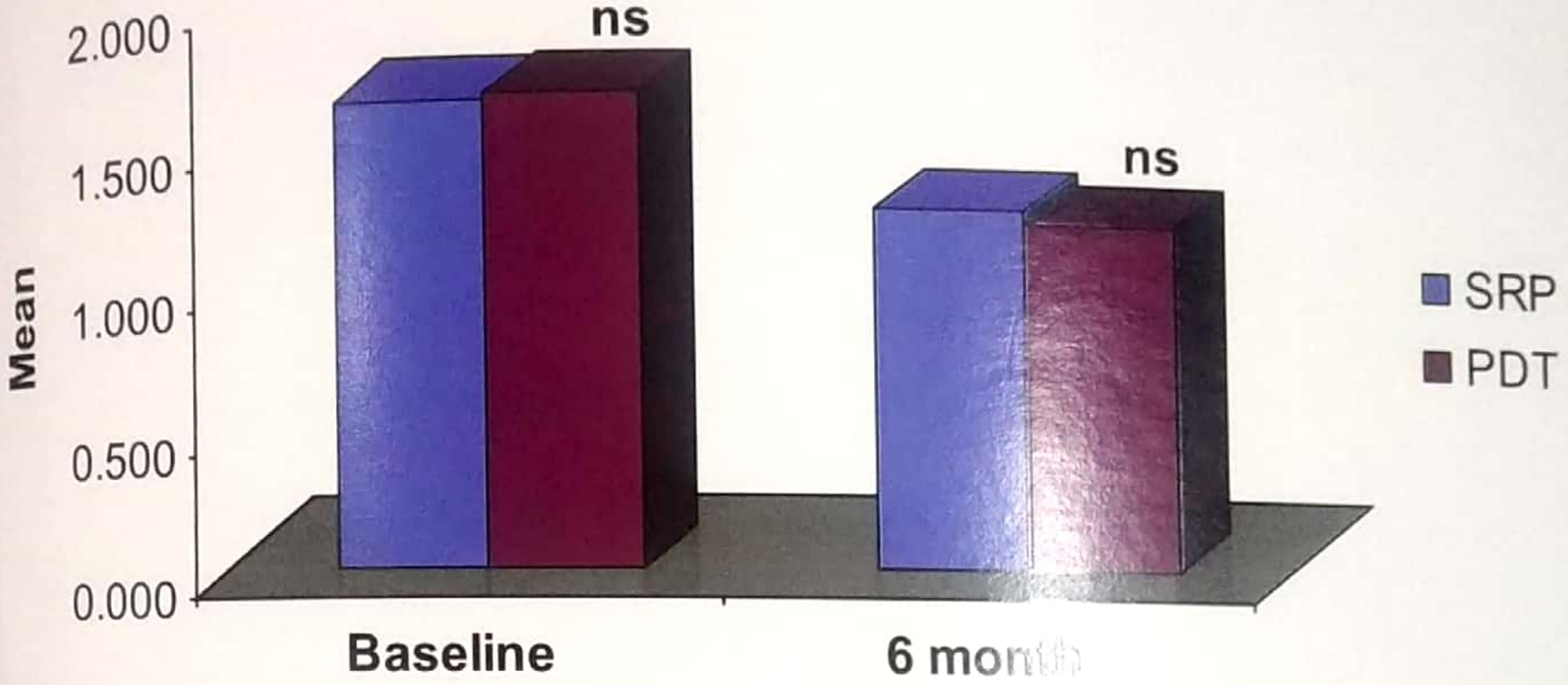
Table 2: Pre and post PI (Mean ± SE) of two groups

Group	Baseline (n=30)	6 month (n=30)	Mean difference	p value
SRP	1.602 ± 0.026	1.227 ± 0.028	0.376	<0.001
PDT	1.625 ± 0.025	1.183 ± 0.023	0.441	<0.001
Mean difference	0.022	0.043	-	-
p value	0.929	0.634		



***p<0.001- as compared to Baseline

Fig. 2. For each group, comparison of mean difference in PI between the periods.



[ns]p>0.05- as compared to SRP

Fig. 3. For each period, comparison of mean difference in PI between the groups.

II. Probing depth

The pre and post PD (mm) of two groups is summarised is summarised in Table 3. In both groups, the mean PD decrease comparatively after the treatment and the decrease was evident slightly higher in PDT than SRP.

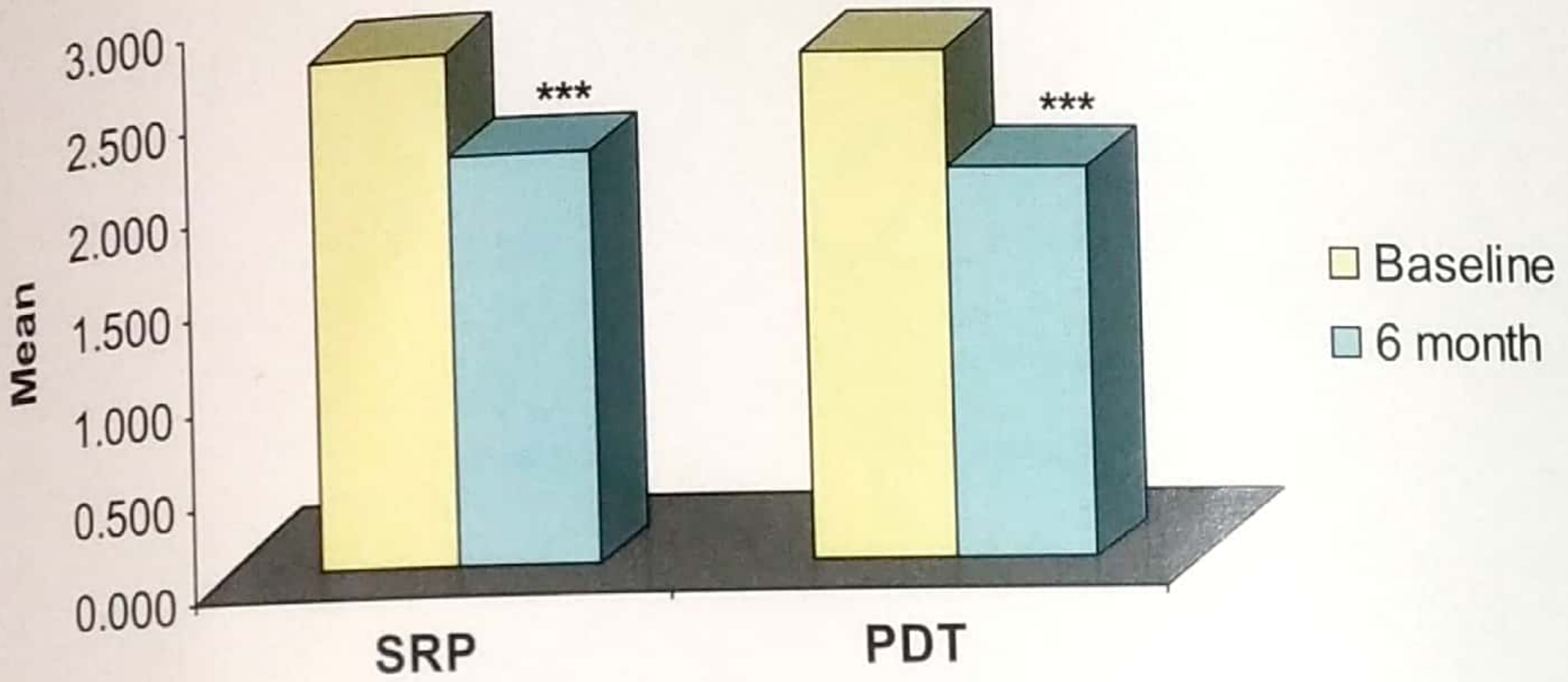
For each group, comparing the mean difference in PD between the periods (baseline vs. 6 month), Tukey test showed significant decrease in PD at 6 month as compared to baseline in both SRP (2.669 ± 0.059 vs. 2.122 ± 0.051, diff=0.547, $p<0.001$) and PDT (2.609 ± 0.057 vs. 2.014 ± 0.060, diff=0.594, $p<0.001$) groups (Table 3 and Fig. 4).

Similarly, for each period, comparing the mean difference in PD between the groups (SRP vs. PDT), Tukey test showed similar PD between the groups at both baseline (2.669 ± 0.059 vs. 2.609 ± 0.057, diff=0.060, p=0.876) and 6 month (2.122 ± 0.051 vs. 2.014 ± 0.060, diff=0.108, p=0.542) i.e. did not differ significantly (Table 3 and Fig. 5).

However, at final evaluation (i.e. the mean change from baseline to 6 month), the decrease in PD of PDT group (22.8%) was found to be 2.3% higher (effect size) as compared to SRP group (20.5%).

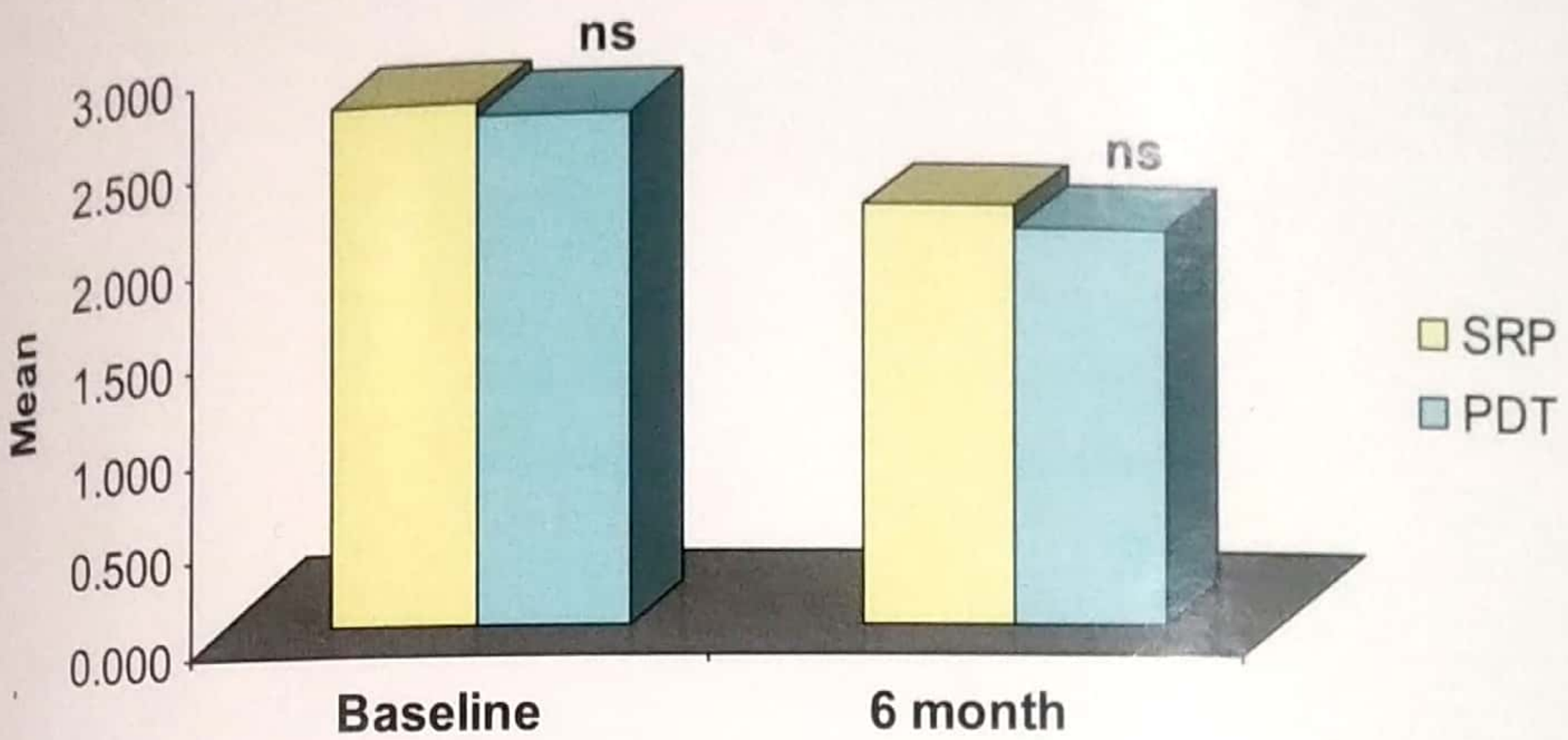
Table 3: Pre and post PD (Mean ± SE) of two groups

Group	Baseline (n=30)	6 month (n=30)	Mean difference	p value
SRP	2.669 ± 0.059	2.122 ± 0.051	0.547	<0.001
PDT	2.609 ± 0.057	2.014 ± 0.060	0.594	<0.001
Mean difference	0.060	0.108	-	-
p value	0.876	0.542		



***p<0.001- as compared to Baseline

Fig. 4. For each group, comparison of mean difference in PD between the periods.



nsp>0.05- as compared to SRP

Fig. 5. For each period, comparison of mean difference in PD between the groups.

III. Clinical attachment level

The pre and post CAL (mm) of two groups is summarised is summarised in Table 4. In both groups, the mean CAL decrease comparatively after the treatment and the decrease was evident slightly higher in PDT than SRP.

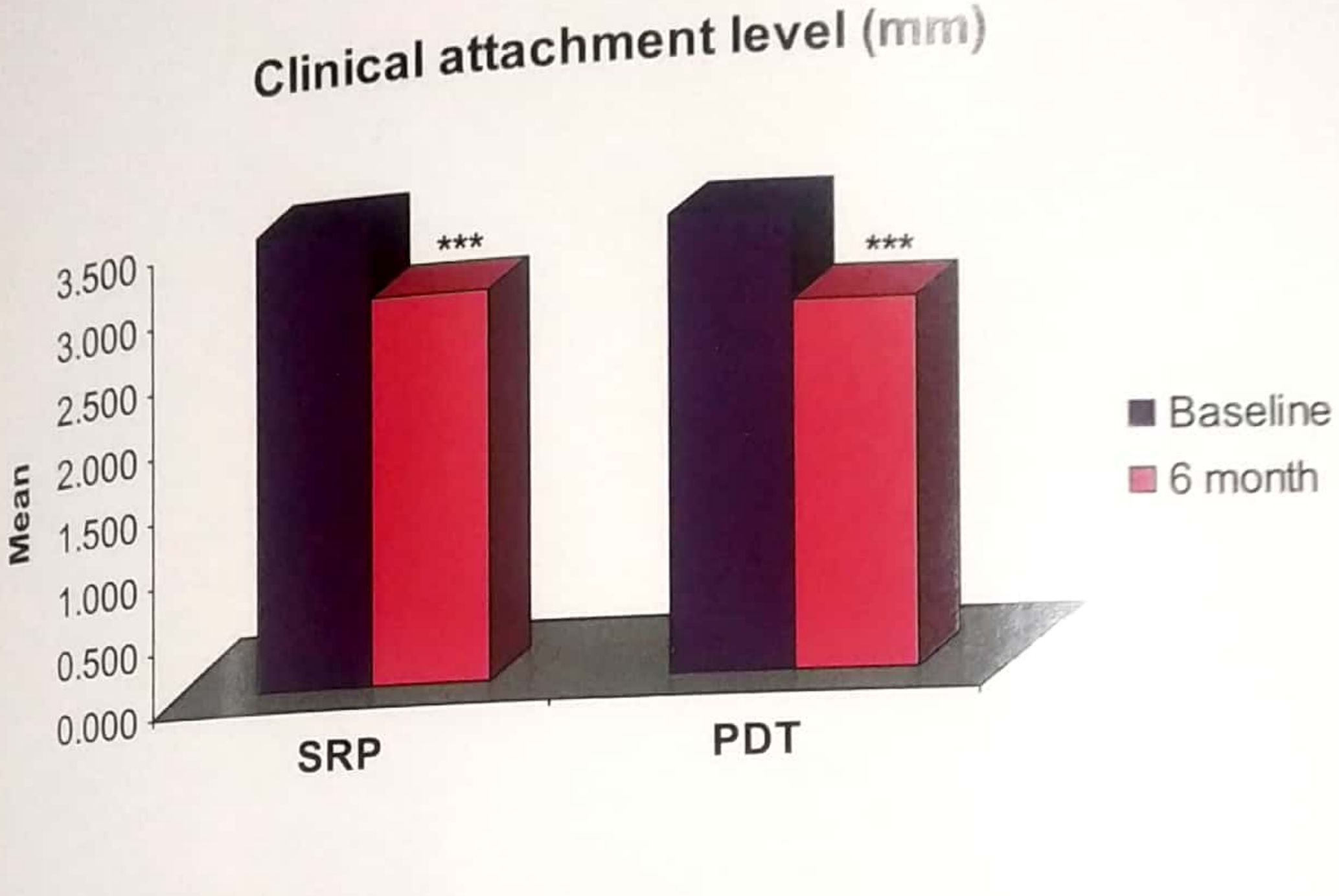
For each group, comparing the mean difference in CAL between the periods (baseline vs. 6 month), Tukey test showed significant decrease in CAL at 6 month as compared to baseline in both SRP (3.397 ± 0.219 vs. 2.858 ± 0.182, diff=0.539, p<0.001) and PDT (3.311 ± 0.213 vs. 2.667 ± 0.183, diff=0.644, p<0.001) groups (Table 4 and Fig. 6).

Similarly, for each period, comparing the mean difference in CAL between the groups (SRP vs. PDT), Tukey test showed similar CAL between the groups at both baseline (3.397 ± 0.219 vs. 3.311 ± 0.213, diff=0.086, p=0.990) and 6 month (2.858 ± 0.182 vs. 2.667 ± 0.183, diff=0.191, p=0.906) i.e. did not differ significantly (Table 4 and Fig. 7).

However, at final evaluation (i.e. the mean change from baseline to 6 month), the decrease in CAL of PDT group (19.4%) was found to be 3.6% higher (effect size) as compared to SRP group (15.9%).

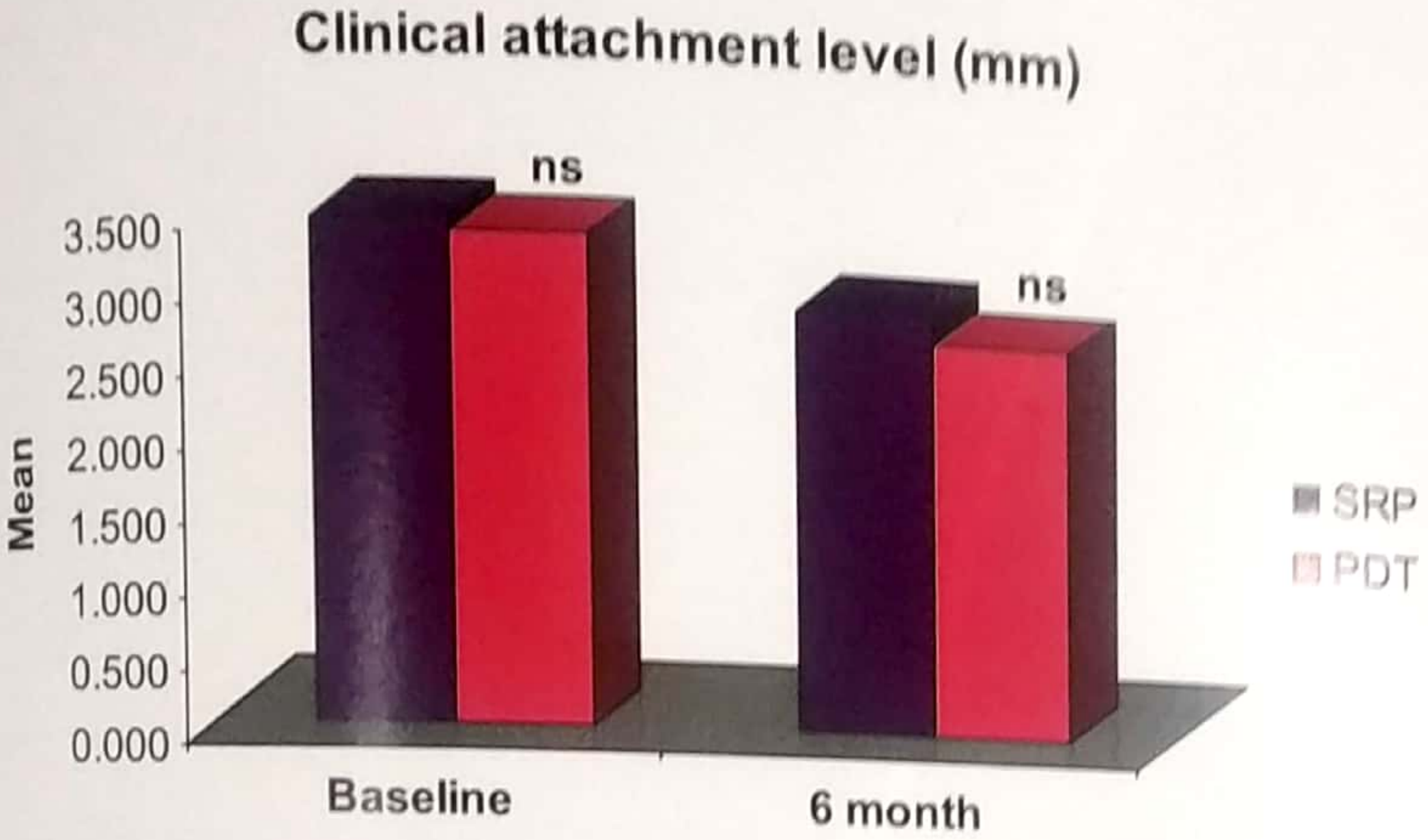
Table 4: Pre and post CAL (Mean ± SE) of two groups

Group	Baseline (n=30)	6 month (n=30)	Mean difference	p value
SRP	3.397 ± 0.219	2.858 ± 0.182	0.539	<0.001
PDT	3.311 ± 0.213	2.667 ± 0.183	0.644	<0.001
Mean difference	0.086	0.191	-	-
p value	0.990	0.906		



***p<0.001- as compared to Baseline

Fig. 6. For each group, comparison of mean difference in CAL between the periods.



[ns]p>0.05- as compared to SRP

Fig. 7. For each period, comparison of mean difference in CAL between the groups.

IV. P. Gingivalis

The pre and post *P. Gingivalis* count (CFU/ml) of two groups is summarised is summarised in Table 5. In both groups, the mean *P. Gingivalis* count decrease comparatively after the treatment and the decrease was evident higher in PDT than SRP.

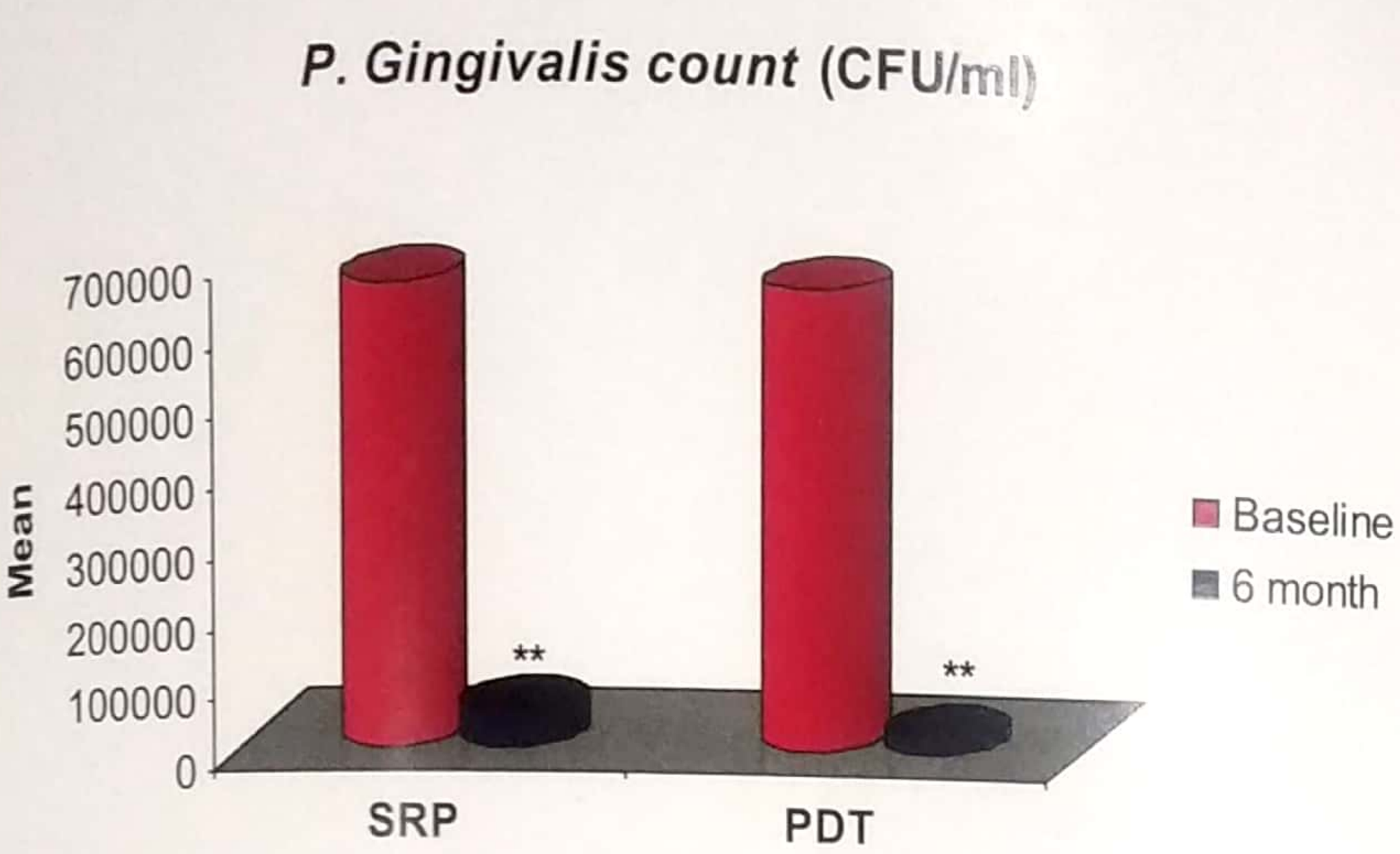
For each group, comparing the mean difference in *P. Gingivalis* count between the periods, Tukey test showed significant decrease in *P. Gingivalis* count at 6 month as compared to baseline in both SRP (653667 ± 177555 vs. 52100 ± 16631, diff=601567, p=0.002) and PDT (646000 ± 157174 vs. 23110 ± 6422, diff=622890, p=0.002) groups (Table 5 and Fig. 8).

Similarly, for each period, comparing the mean difference in *P. Gingivalis* count between the groups, Tukey test showed similar *P. Gingivalis* count between the groups at both baseline (653667 ± 177555 vs. 646000 ± 157174, diff=7667, p=1.000) and 6 month (52100 ± 16631 vs. 23110 ± 6422, diff=28990, p=0.998) i.e. did not differ significantly (Table 5 and Fig. 9).

However, at final evaluation (i.e. the mean change from baseline to 6 month), the decrease in *P. Gingivalis* count of PDT group (96.4%) was 4.4% higher (effect size) as compared to SRP group (92.0%).

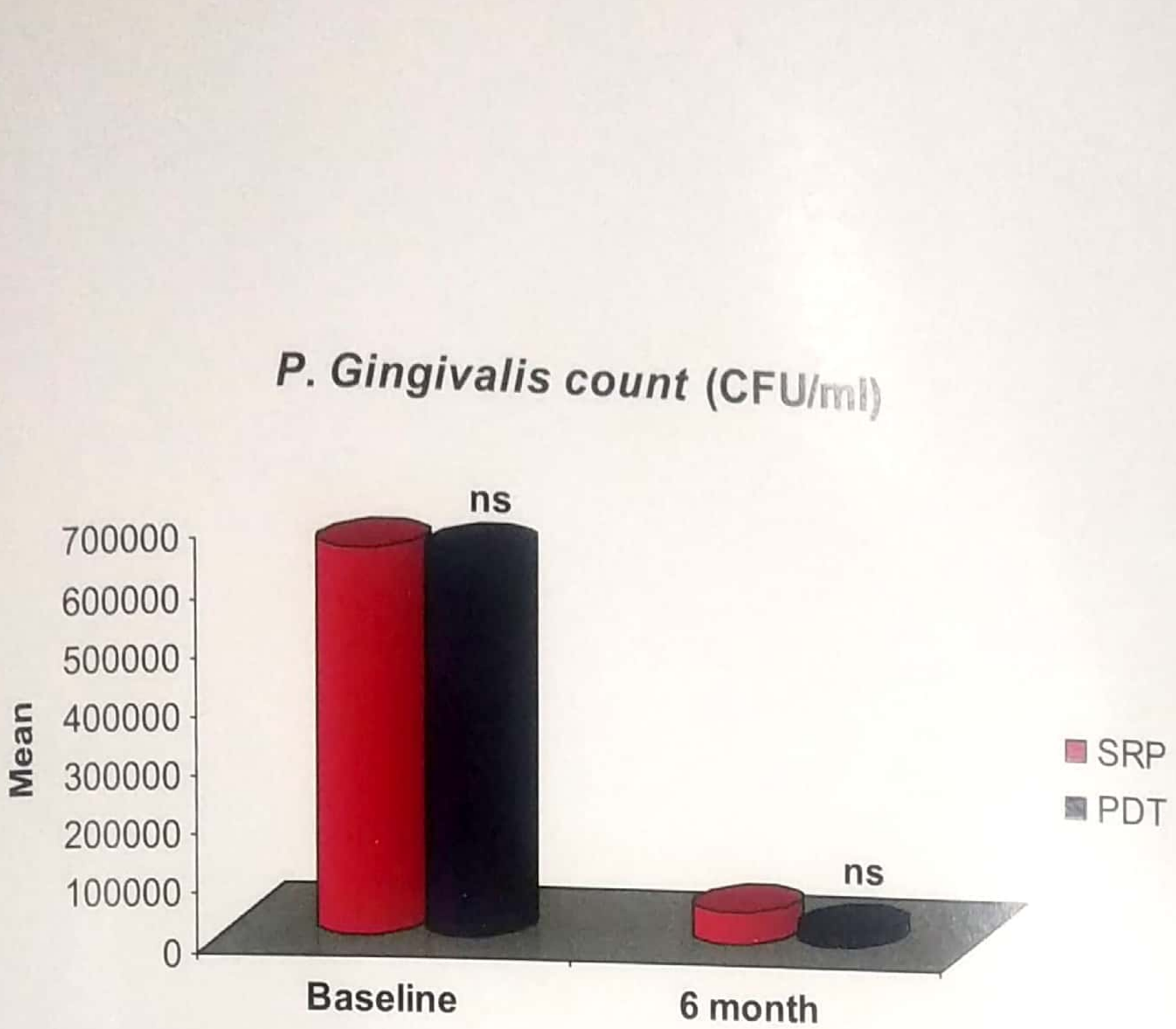
Table 5: Pre and post *P. Gingivalis* count (Mean ± SE) of two groups

Group	Baseline (n=30)	6 month (n=30)	Mean difference	p value
SRP	653667 ± 177555	52100 ± 16631	601567	0.002
PDT	646000 ± 157174	23110 ± 6422	622890	0.002
Mean difference	7667	28990	-	-
p value	1.000	0.998		



**p<0.01- as compared to Baseline

Fig. 8. For each group, comparison of mean difference in *P. Gingivalis* count between the periods.



[ns]p>0.05- as compared to SRP

Fig. 9. For each period, comparison of mean difference in *P. Gingivalis* count between the groups.

Statistical analysis

Data were summarised as Mean ± SE (standard error of the mean). Groups were compared by repeated measures two way analysis of variance (ANOVA) using general linear models (GLM) and the significance of mean difference within (intra) and between (inter) the groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. A two-tailed (α=2) $p<0.05$ was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0).

Discussion

Periodontitis is a multifactorial disease that is associated with loss of the supporting tissues around the tooth.[56] A major objective of periodontal therapy is to remove soft and hard, supra- and subgingival deposits from the root surface to stop disease progression.[57]The effect of scaling and root planing (SRP) on the subgingival microflora has been summarized in numerous reviews.[58] SRP remains the gold standard in maintaining chronic periodontal sites by reducing and shifting the microbial load to a more biological compatible microflora and this in turn improves the clinical parameters. Unfortunately these improvements are short lived (approximately 12 weeks) due to repopulation of the biofilm matrix by periopathogens which may result in inflammation and/or loss of attachment and alveolar bone.[59] Furthermore, SRP requires a certain level of skill and time, has limited access in challenging areas such as deep pockets and furcations and the potential for unnecessary removal of root substance. Therefore, complete subgingival pocket disinfection is difficult. Another difficulty lies with the fact that no single microorganism has been identified as the etiologic microorganism(s) causing disease.[60]This uncertainty coupled with the limitations of SRP makes treating chronic periodontal sites challenging.

The adjunctive use of either systemic or localized antibiotics has been extensively tested for their therapeutic efficacy. Systematic reviews and other studies have concluded that the use of systemically administered adjunctive antibiotics with or without SRP and/or surgery appeared to provide greater clinical improvement. While these reviews indicate, on average, antibiotics do contribute to a statistically significant improvement in periodontal clinical indices, it is still unclear on the magnitude of the added benefit, the optimal dosage, the optimal agent(s), the frequency of application, the

identification of the patients that would most benefit, patient compliance and the increasing concern of antibiotic overuse and resistance.[61]

The above mentioned concerns have fostered research into novel approaches to manage bacterial infections. Photodynamic Therapy (PDT) has been extensively investigated *in vitro* with a lesser extend *in vivo* for the eradication of oral bacteria. *In vitro* research established that several associated periodontopathogens in the subgingival biofilms like *P. gingivalis, Fusobacterium nucleatum, Staphylococcus species* are efficiently eradicated by photodynamic treatment, both in aqueous suspension and in biofilm.[62]

PDT utilizes low power lasers with appropriate wavelength to kill microorganisms treated with a photosensitizer drug, preferably a dye. The knowledge of the preferred uptake and accumulation of some dyes (mostly porphyrins) into tumor tissues stimulated the introduction of PDT into clinical practice. PDT is based on the principle that a photoactivable substance (the photosensitizer) binds to the target cell and can be activated by light of a suitable wavelength.[65] During this process, free radicals are formed (among them is singlet oxygen), which then produce an effect that is toxic to the cell. To have a specific toxic effect on bacterial cells, the respective photosensitizer needs to have selectivity for prokaryotic cells. Although several authors have reported the possibility of a lethal photosensitization of bacteria *in vivo* and *in vitro*, others have pointed out that Gram negative bacterial species, due to their special cell wall, are largely resistant to PDT.[66]

PDT involves three components: Light, a photosensitizer (PS) and oxygen. The photosensitizer is administered to the patient, and upon irradiation with light of a specific

wavelength, the photosensitizer undergoes a transition from a low energy ground state to an excited singlet state. Subsequently, the photosensitizer may decay back to its ground state with the emission of fluorescence or may undergo a transition to a higher energy triplet state.[67] The triplet state photosensitizer can react with biomolecules in three different pathways - type I, type II and type III.[68]

Type I reaction involves electron - transfer reactions between the excited state of the photosensitizer and an organic substrate molecule of the cells, producing free radicals. These free radical species are highly reactive and interact with endogenous molecular oxygen to produce highly reactive oxygen species, such as superoxide, hydroxyl radicals and hydrogen peroxide, which are harmful to cell membrane integrity, causing irreparable biological damage.

In type II reaction, the triplet state photosensitizer reacts with oxygen to produce an electronically excited and highly reactive state of oxygen, known as singlet oxygen ($^1O_2$) which can interact with a large number of biological substrates inducing oxidative damage on the cell membrane and cell wall. Microorganisms that are killed by singlet oxygen include viruses, bacteria and fungi. Singlet oxygen has a short lifetime in biological systems and a very short radius of action (0.02 mm). Hence, the reaction takes place within a limited space, leading to a localized response; thus making it ideal for application to localized sites without affecting distant cells or organs. Thus, the type II reaction is accepted as the major pathway in microbial cell damage.[69]

Type III reaction is a unique reaction because it is oxygen independent. These reactions require either high concentration of the photosensitizer or a deaerated system, in order to

bypass the reaction with oxygen. Under anaerobic condition, radicals are generated and these can subsequently react to cause cell death.[70]

PDT is widely considered as an adjunctive to conventional mechanical therapy. The two reasons that justify the use of PDT in the field of periodontology are technical simplicity and effective bacterial eradication. Antimicrobial PDT not only kills the bacteria, but may also lead to the detoxification of endotoxins such as lipopolysaccharide. These lipopolysaccharides treated by PDT do not stimulate the production of proinflammatory cytokines by mononuclear cells. Thus, PDT inactivates endotoxins by decreasing their biological activity.

The adjunctive use of PDT to scaling and root planing results in greater clinical attachment level gains, reduction in bleeding on probing and probing pocket depths. Also PDT has advantages such as reducing the treatment time, destruction of bacteria, inactivation of endotoxins, unlikely development of resistance by the target bacteria and no damage to the adjacent host tissues. In addition, the use of PDT in furcation involvement shows some advantages such as reduced need for flap procedures and shorter treatment time with lack of microflora disturbance in other sites of oral cavity.

Clinical studies over the years have evaluated the potential of PDT in treatment of periodontal diseases but there are certain confounding factors that may inhibit or diminish the bactericidal efficiency of PDT. These factors include the type of photosensitizer (PS) and the light source used in the treatment procedure.

The photosensitizer selection is crucial to achieve a successful photochemical reaction. It is imperative that the chosen PS for treatment is retained by target cells (bacteria in this

case), absorbs at the desired wavelength of light with a high extinction coefficient and high quantum yield of singlet oxygen.

The structural characteristics of phenothiazinum-based photosensitizers (PhBPs) like toluidine blue-O allow them to be highly suited for PDT by exhibiting significant singlet oxygen yields and absorbing at therapeutic wavelengths (620-700nm).[72] Chan and Lai in a an *in vitro* study revealed that oral microorganisms were subjected to cell death with a 665nm diode laser, at 100 mW with 60 seconds irradiance while incorporated with 0.01% wt/vol toluidine blue-O dye.[73] Furthermore the cationic nature of phenothiazine has been proven to penetrate deeper in the plaque biofilm and increase the killing rate.[74] Although most studies demonstrated a log reduction in bacteria, a confocal laser scanning micrograph (CLSM) of a biofilm after exposure to phototoxic mechanism revealed that in some of the biofilm stacks, lethal photosensitization occurred predominantly in the outer layers of the stack leaving some of the innermost bacteria alive[75] which may allow for bacterial recolonization of certain pathogens such as, *A. actinomycetemcomitans* and *P. gingivalis* which are known to invade host tissue cells and elude the effects of nonsurgical periodontal therapy(NSPD).[76]

The effectiveness of a light source for PDT depends but is not limited to, spectral irradiance, tissue transmission and photosensitizer absorption.[77] Chan and Lai demonstrated *in vitro* that both wavelength and energy density are important factors in achieving optimal bacterial kill.[78] Most photosensitizers are activated by red light between 630 and 700 nm, corresponding to a penetration depth from 0.5 cm to 1.5 cm.[79] This limits the depth of necrosis. Currently, the light source applied in photodynamic therapy are those of helium - neon lasers (633 nm), gallium - aluminum - arsenide diode

lasers (630-690, 830 or 906 nm) and argon laser (488-514 nm).[80] In the present study, PDT was performed with a diode laser with power settings at 630nm wavelength, 2mW output and continuous mode irradiated with toluidine blue-O (1mg/ml) solution as a photo sensitizer.

In addition to clinical evaluation, microbiological analysis was conducted to answer the question whether topical application of PDT as an adjunct to SRP can improve the effectiveness of the treatment.

The bactericidal effect of antimicrobial photodynamic therapy on periodontal pathogens has been demonstrated in several studies. Bhatti et al. demonstrated that the optimal concentration of toluidine blue O to kill P. gingivalis was 12.5 mg/ml with helium–neon laser irradiation. In addition, they revealed, by transmission electron microscopic examination, that the bactericidal effect of light-activated toluidine blue O against P. gingivalis was caused by disruption of the outer membrane proteins of those bacteria.[81] Recently, Qin et al. investigated the optimal parameters required for effective antimicrobial photodynamic therapy-induced killing of supragingival periodontal pathogens using the combination of different toluidine blue O concentrations and laser-irradiation energies and reported that diode laser irradiation at 12 J/cm2 with 1 mg/ml of toluidine blue O was the most effective option.[82] Chan and Lai showed that in the presence of methylene blue, the wavelengths of 632.8 nm (helium–neon laser) and 620 and 830 nm (diode laser) had a high bactericidal effect on periodontal pathogens. In black-pigmented bacteria such as P. gingivalis and Prevotella species, the endogenous porphyrins present on the bacteria may also act as a photosensitizer.[83] In this study, the

bactericidal effect of PDT on p.gingivalis was investigated and the confirmatory analysis was done using real-time PCR (Polymerase Chain Reaction) using specific primers.

The results of the current study showed that the mean PD decreased comparatively six months after treatment and the decrease was evident slightly higher in PDT group than in SRP group. At final evaluation (i.e. the mean change from baseline to 6 month), the decrease in PD of PDT group (22.8%) was found to be 2.3% higher (effect size) as compared to SRP group (20.5%).However, inter-group differences were not significant, consistent with the results of a study of Polansky et al, who did not report significant differences in probing pocket depth between the SRP group and the PDT–SRP group three months after baseline, although the PD reduction in the test group was higher than that in the control group.[84] Similar results were reported by Yilmaz et al after 3 and 6 months.[85] Anderson et al and Christodoulides et al also reported significant decrease in PD with the use of PDT as an adjunct to SRP.[86-87]

One of the reasons for insignificant inter-group PD reduction is good plaque control by the patients. In this study, except for 3 patients with poor oral hygiene, the remaining patients had good plaque control, with a lower plaque scores. Therefore, administration of PDT in patients with good plaque control and healthy patients with no systemic disease can be questioned as it could not have additional benefits over SRP. As a result, monitoring the application of PDT can be focused on patients with compromised condition and also in more severe forms of periodontitis, like aggressive periodontitis or periodontitis modified by systemic factors.

In terms of clinical attachment level (CAL), the decrease in CAL of PDT group (19.4%) was found to be 3.6% higher (effect size) as compared to SRP group

(15.9%) after six months.These results were consistent with the results obtained by Yilmaz et al, Andersen et al, Christodoulides et al, Braun et al. and Chondros et al.[85-89] The reason for these results can be explained on the basis that, although physical plaque biofilms removal by scaling and root planing is an essential part of periodontal therapy, complete removal of plaque and calculus is not always possible especially in inaccessible sites such as deeper pockets and furcation areas. Thus, the better clinical outcome in the PDT group compared to the SRP group in the present study might be attributed partly to the effect of photosensitization on viability of periodontal pathogens in these sites.

Also, at 6 months, PDT with SRP resulted in significant improvement in plaque scores as compared to SRP alone.The decrease in PI of PDT group (27.2%) was found to be 3.7% higher (effect size) as compared to SRP group (23.4%). These findings are in agreement with the results found by Yilmaz et al[85], Andersen et al[86], Braun et al[88], Chondros et al[89] and also in a recent controlled clinical trial by Qadri and associates in which they found that treatment with low-level laser irradiation as an adjunct to conventional SRP in periodontal subjects significantly reduced periodontal gingival inflammation.[90] In that study, gingival inflammation was evaluated through a sampled volume of gingival crevicular fluid (GCF) that was analyzed for elastase activity, interleukin-1b and metalloproteinase-8 (MMP-8). The decrease in the total volume of GCF was significantly greater in the laser group, and the difference in measured MMP-8 approached significance.

Since higher plaque scores in multiple examinations with increasing probing depth is correlated with the progression of periodontitis, controlling gingival inflammation by maintaining pockets at least in a healthy condition can be a successful

treatment option, especially in patients in which periodontal surgery is contraindicated. The positive results of PDT on plaque score reduction may provide some advantages to achieve this goal. No adverse reaction was observed from PDT. It is of utmost importance because it can facilitate its use in clinical practice. Moreover, it does not have some of the disadvantages of systemic antibiotics, such as the emergence of resistant bacteria or GI disturbances.

Considering that periodontal disease is caused by disequilibrium between dental plaque and host defense system, monitoring the number of pathogens like *P. gingivalis* is necessary before and after treatment.[91] Detection of pathogens is influenced by detection methods. Real-time polymerase chain reaction by double fluorescent probes provides precise quantification of bacteria and is one of the most accurate technologies in this field.

In the current study, *P. gingivalis* count decreased significantly after six months, with no significant differences between the two groups. The results showed the decrease in *P. Gingivalis* count of PDT group (96.4%) was 4.4% higher (effect size) as compared to SRP group (92.0%). These results are similar to the results obtained by Chondros et al in which they found that *P. gingivalis* count decreased three months after treatment but inter group differences were not significant.[92] Also, in a study by Polansky et al, *P. gingivalis* counts decreased in both PDT and SRP groups, without significant inter-group differences.[93] Christodoulides et al and Cappuyns et al reported similar trends in their studies.[94]

Although the susceptibility of periodontal pathogens to photodynamic therapy has been shown in vivo, its bactericidal effects in human studies is not well established because

these studies have used conventional PCR which does not quantify the number of bacteria, which is one of the limitations of conventional PCR compared to real time PCR used in this study.

Within the limitations of the current study, subgingival application of photodynamic therapy combined with scaling and root planing shows greater reduction in pocket depth and plaque scores and greater gain in clinical attachment level. It is suggested that further longitudinal studies with a larger sample size are required to confirm the findings of this study and implement the use of photodynamic therapy in daily clinical practice.

Conclusion

The results of present study revealed that:

- Among the periodontal clinical parameters, the clinical attachment level showed a greater gains in PDT Group (patients who were treated with photodynamic therapy in addition to scaling and root planing). Further, the decrease in probing pocket depth and plaque scores was greater in the group treated with photodynamic therapy.
- In addition, the microbiological analysis done to determine the bactericidal effect of PDT on *P. gingivalis* revealed significant decrease in the count of the bacteria at baseline and six months post treatment.

It can be concluded that photodynamic therapy is an interesting and effective therapeutic approach towards the treatment of periodontitis. Within its limits, the present study demonstrated that adjunctive PDT resulted in reduction of pocket depth, gain in clinical attachment level and decrease in the count of *P. gingivalis.*

Conventional treatment such as scaling and root planing for removal of plaque and calculus are the basis of any periodontal treatment, are upgraded by antimicrobial photodynamic therapy mainly due to its powerful decontaminating effect.

Photodynamic therapy induces a significant reduction of bacteria without any invasive effects. What is more, it enables a high level of disinfection of the oral cavity, preventing attacks by bacteria and thus eliminating the direct cause of the infection, stopping progress of the disease and extending the prognosis for the tooth involved.

Summary

Chronic periodontitis (CP) is a chronic inflammatory response to the accumulation of microbial plaque and calculus on the root surface of the tooth; this condition leads to breakdown of the surrounding periodontal tissues. The gold standard for the non-surgical treatment of periodontal disease is scaling and root planing (SRP). In SRP, the removal of supragingival and subgingival biofilms along with the diseased root surface is facilitated using hand instruments and ultrasonic scalers. However, complete removal of the bacterial biofilm and their endotoxins in deeper areas of the pockets and furcation sites is often difficult to achieve with both methods.

Recently, photodynamic therapy (PDT) has been introduced in periodontal therapy in an attempt to improve the effectiveness and efficiency of root surface debridement and bacterial elimination and to overcome the above mentioned limitations of SRP.

PDT involves three components: light, a photosensitizer, and tissue oxygen. Laser is the preferred source of light for photodynamic therapy because it emits coherent, monochromatic, intense and unidirectional light while methylene blue and toluidine blue O are very effective photosensitizing agents for the inactivation of both gram-positive and gram-negative bacteria. Upon irradiation, the photosensitizer undergoes a transition from a low energy ground state to a higher energy triplet state, which can then react with biomolecules to produce free radicals (type I reaction), or with molecular oxygen to produce highly reactive singlet oxygen (type II reaction),leading to cell death.

The numerous in vitro and in vivo studies have clearly demonstrated its effective and efficient bactericidal effect and is extensively studied in periodontics due to its technical

simplicity and its ability to eradicate bacteria, the causative agents behind occurrence of periodontal diseases.

The present study was conducted to comparatively evaluate the efficacy of photodynamic therapy as an adjunct to SRP in the treatment of chronic periodontitis patients with the help of clinical and microbiological parameters.

A total of 30 subjects reporting to the OPD of Department of Periodontics aged between 25-50 years were selected for the study. The study protocol was explained to all patients and those who fulfilled the criteria were enrolled in the study. After clinical and radiographic assessment, two quadrants were randomly selected in each subject which would receive either scaling and root planing along with photodynamic therapy (PDT GROUP) or scaling and root planing alone (SRP GROUP). Plaque index (PI), probing depth (PD) and clinical attachment level (CAL) were recorded for both the groups.

Subgingival plaque samples were collected for microbiological analysis from the selected PDT and SRP sites. After full mouth scaling and root planing, PDT was performed with a diode laser with power settings at 630nm wavelength, 2mW output and continuous mode irradiated with toluidine blue-O (1mg/ml) solution as a photosensitizer in one of the quadrant (PDT GROUP) while the other selected quadrant acted as a placebo (SRP GROUP). The photosensitizer was applied to the bottom of the periodontal pocket with the help of an insulin syringe. After 3 minutes of action the photosensitizer was rinsed with saline and exposed to diode laser for 30 seconds at each site. After the procedure, oral hygiene instructions were given to the patients and were advised to visit the clinic regularly for evaluation.

After six months, plaque samples were collected from the same sites and clinical parameters were re-recorded. After analysis of the data from the baseline and six months after treatment, the following results were found.

The mean plaque index (PI), probing depth (PD) and clinical attachment level (CAL) decreased comparatively after the treatment and the decrease was evident slightly higher in PDT than SRP group. At final evaluation (i.e. the mean change from baseline to 6 month), the decrease in PI of PDT group (27.2%) was found to be 3.7% higher (effect size) as compared to SRP group (23.4%),), the decrease in PD of PDT group (22.8%) was found to be 2.3% higher (effect size) as compared to SRP group (20.5%) and the decrease in CAL of PDT group (19.4%) was found to be 3.6% higher (effect size) as compared to SRP group (15.9%).

The microbiological analysis of *P. gingivalis* was done for evaluating the bactericidal effect of PDT and at final evaluation (i.e. the mean change from baseline to 6 month), the decrease in *P. Gingivalis* count of PDT group (96.4%) was 4.4% higher (effect size) as compared to SRP group (92.0%).

So, it can be concluded that, within the limitations of the study, subgingival application of photodynamic therapy combined with scaling and root planing shows greater reduction in pocket depth and plaque scores and greater gain in clinical attachment level. Conventional treatment such as scaling and root planing for removal of plaque and calculus are the basis of any periodontal treatment, are upgraded by antimicrobial photodynamic therapy mainly due to its powerful decontaminating effect. It is suggested that further longitudinal studies with a larger sample size are required to confirm the

findings of this study and implement the use of photodynamic therapy in daily clinical practice.

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Appendices

APPENDIX-1

Institutional Ethical Committee Letter

Babu Banarasi Das College of Dental Sciences
(A Faculty of Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the 3rd Institutional Ethics Sub Committee Meeting.

IEC Code: 28 **BBDCODS/ 28 /2015**

Title of the Project: Photodynamic Therapy As An Adjunct To SRP In The Treatment Of Chronic Periodontitis: A Clinical And Microbiological Study.

Principal Investigator: Dr. Rajeev Kumar Singh **Department:** Periodontology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow

Type of Submission: New, MDS Protocol

Dear Dr. Rajeev Kumar Singh

The Institutional Ethics Sub Committee meeting was held on 09-01-2015. The sub committee comprises following four members :

1.	Dr. Amrit Tandon Member	Prof. & Head, Deptt. of Prosthodontics BBDCODS, Lucknow.
2.	Dr. Jiji George Member	Prof., Deptt. of Oral Pathology & Microbiology, BBDCODS, Lucknow.
3.	Dr. Ashish Saini Member	Reader, Department of Periodontology, BBDCODS, Lucknow.
4.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Deptt. of Biochemistry, BBDCODS, Lucknow.

The committee reviewed and discussed your submitted documents of the research study in the meeting. The proposal was reviewed and thoroughly revised.

Decisions of the IEC : As per the recommendations I.E.C. has taken following decisions for the current protocol of study "Photodynamic Therapy As An Adjunct To SRP In The Treatment Of Chronic Periodontitis: A Clinical And Microbiological Study."

The committee approved the above proposal from ethics point of view.

Lakshmi Bala 19/2/15

(Dr. Lakshmi Bala)
Member-Secretary IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

Forwarded by:

(Dr. Vivek Govila)
Dean

DEAN
BBD College of Dental Sciences
BBD University
Faizabad Road Lucknow-226028

APPENDIX-2

CONSENT FORM

Title of the Study .....

Study Number.....

Subject's Full Name.....

Date of Birth/Age .....

Address.....

Phone no. and e-mail address.....

1. I confirm that I have read and understood the Participant Information Document dated .....for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.

2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.

4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [ ] No [ ] Not Applicable [ ]**

6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:.....

Signatory's Name.....

Date .....

Signature of the Investigator.....

Date.....

Study Investigator's Name.....

Date.....

Signature of the witness.....

Date.....

Name of the witness.....

Received a signed copy of the PID and consent form

Signature/thumb impression of the subject or legally acceptable representative

Date.....

APPENDIX-3
CASE HISTORY PROFORMA

Date:

Name: **Age:** **OPD no:**

Sex:

Address: **Mobile no.:** **Occupation:**

CHIEF COMPLAINT (S):

PAST MEDICAL AND DENTAL HISTORY

INDICES: (At baseline)

A. PLAQUE INDEX:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

B. PROBING POCKET DEPTH:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

C. CLINICAL ATTACHMENT LEVEL:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

STUDY GROUPS:

PDT GROUP (QUADRANT NO.):

SRP GROUP (QUADRANT NO.):

SUBGINGIVAL SAMPLE COLLECTION:

INDICES: (After 6 months)

A. PLAQUE INDEX:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

B. PROBING POCKET DEPTH:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

C. CLINICAL ATTACHMENT LEVEL:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

SUBGINGIVAL SAMPLE COLLECTION:

Patient consent and signature

I am aware of the treatment that is being carried out on me and willing to be part of the study.

ANNEXURE – IV

OBSERVATIONS

GROUP I: SRP

SNO	PLAQUE INDEX		PROBING DEPTH (mm)		CLINICAL ATTACHMENT LEVEL (mm)		P.Gingivalis count (CFU/ml)	
	Baseline	6 month	Baseline	6 month	Baseline	6 month	Baseline	6 month
1	1.532	1.231	2.714	2.212	2.06	1.6	2000000	20000
2	1.756	1.282	2.6	2.08	1.82	1.68	20000	1000
3	1.82	1.42	2.531	1.98	2.25	1.88	3000000	300000
4	1.89	1.37	2.822	2.36	2.46	1.96	300000	10000
5	1.562	1.17	2.763	2.23	4.76	3.98	30000	3000
6	1.51	0.98	2.33	1.89	3.736	2.536	300000	3000
7	1.367	1.052	2.602	2.013	4.35	3.82	300000	10000
8	1.512	1.124	2.593	2.026	4.736	3.82	300000	100000

9	1.704	1.217	2.13	1.93	4.75	3.96	200000	100000
10	1.642	1.285	2.672	2.012	4.72	3.96	200000	10000
11	1.592	1.296	2.618	2.025	4.73	3.89	30000	1000
12	1.61	1.19	2.86	2.26	4.65	3.87	200000	20000
13	1.53	1.02	2.82	2.12	4.42	3.58	20000	1000
14	1.643	1.243	3.642	3.013	4.482	4.028	300000	300000
15	1.58	1.25	3.282	2.613	4.502	4.122	300000	10000
16	1.224	0.986	2.826	1.816	2.231	1.732	30000	3000
17	1.571	1.362	2.516	1.762	3.223	2.873	300000	3000
18	1.76	1.48	2.353	1.89	2.1	1.98	300000	10000
19	1.5	1.236	2.82	1.97	2.02	1.89	300000	100000
20	1.607	1.251	2.68	1.98	1.98	1.37	200000	100000
21	1.536	1.116	2.72	2.42	2.06	1.89	200000	10000
22	1.732	1.53	2.36	2.06	2.16	1.97	30000	1000
23	1.82	1.42	2.53	1.98	2.26	1.87	200000	20000

							0	
24	1.51	0.98	2.34	1.89	3.76	2.56	20000	1000
25	1.704	1.217	2.13	1.93	4.75	3.96	300000 0	300000
26	1.61	1.19	2.86	2.26	4.65	3.87	300000	10000
27	1.58	1.25	3.282	2.613	3.503	3.3122	30000	3000
28	1.763	1.483	2.353	1.893	2.105	1.98	300000	3000
29	1.535	1.116	2.72	2.42	2.06	1.98	300000	10000
30	1.367	1.052	2.602	2.013	4.63	3.82	300000	100000

GROUP II: SRP + PDT

SNO	PLAQUE INDEX		PROBING DEPTH (mm)		CLINICAL ATTACHMENT LEVEL (mm)		P.Gingivalis count (CFU/ml)	
	Baseline	6 month	Baseline	6 month	Baseline	6 month	Baseline	6 month
1	1.62	1.13	2.65	2.05	2.14	1.56	200000	10000
2	1.645	1.282	2.56	2.02	1.73	1.46	200000	10000

3	1.87	1.37	2.522	2.22	2.3	1.96	200000	10000
4	1.82	1.47	2.736	2.224	2.47	1.73	20000	100
5	1.756	1.23	2.226	2.116	4.665	3.23	200000	100000
6	1.642	1.121	2.32	1.73	3.63	2.46	300000	10000
7	1.704	1.217	2.53	1.84	4.52	3.72	200000	1000
8	1.505	1.103	2.53	1.96	4.63	3.71	200000	10000
9	1.366	1.116	2.14	1.83	4.72	3.82	200000	10000
10	1.523	1.126	2.576	2.06	4.665	3.865	200000	100000
11	1.572	1.196	2.73	2.06	4.65	3.6	300000	10000
12	1.61	1.196	2.865	2.263	4.55	3.9	200000	10000
13	1.43	0.98	2.73	2.13	4.37	3.6	200000	10000
14	1.624	1.223	3.56	3.1	2.76	2.03	200000	10000
15	1.76	1.24	3.22	2.46	4.466	4.054	20000	100
16	1.33	0.96	2.72	1.62	2.116	1.626	200000	100000

17	1.52	1.26	2.517	1.601	3.166	2.736	300000	10000
18	1.663	1.323	2.45	1.63	2.06	1.73	20000	1000
19	1.73	1.13	2.72	1.73	2.02	1.67	200000	10000
20	1.563	1.243	2.502	1.42	1.89	1.46	300000	10000
21	1.632	1.006	2.623	2.23	2.05	1.76	200000	100000
22	1.716	1.443	2.26	2.03	2.17	1.79	300000	10000
23	1.87	1.07	2.522	2.126	2.37	1.96	200000	10000
24	1.642	1.121	2.32	1.73	3.63	2.46	200000	10000
25	1.366	1.116	2.143	1.83	4.723	3.732	200000	10000
26	1.74	1.04	2.76	2.06	4.554	3.98	20000	100
27	1.56	1.24	3.22	2.46	4.46	4.01	200000	100000
28	1.72	1.32	2.45	1.63	2.06	1.36	300000	10000
29	1.532	1.006	2.623	2.23	2.05	1.76	200000	1000
30	1.704	1.217	2.534	2.043	3.743	3.276	200000	10000

ANNEXURE – V

Formula used for the analysis

Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Standard Deviation and Standard Error

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

and SE (standard error of the mean) is calculated as

$$SE = \frac{SD}{\sqrt{n}}$$

Where, n= no. of observations

Analysis of Variance

Analysis of variance (ANOVA) is used when we compare more than two groups simultaneously. The purpose of one-way ANOVA is to find out whether data from several groups have a common mean. That is, to determine whether the groups are actually different in the measured characteristic. One way ANOVA is a simple special case of the linear model. For more than two independent groups, simple parametric ANOVA is used when variables under consideration follows Continuous exercise group distribution and groups variances are homogeneous otherwise non parametric alternative Kruskal-Wallis (H) ANOVA by ranks is used. The one way ANOVA form of the model is

$$Y_{ij} = \alpha_{.j} + \varepsilon_{ij}$$

Where;

- Y_{ij} is a matrix of observations in which each column represents a different group.

- α_j is a matrix whose columns are the group means (the "dot j" notation means that α applies to all rows of the j^{th} column i.e. the value α_{ij} is the same for all i).

- ε_{ij} is a matrix of random disturbances.

The model posits that the columns of Y are a constant plus a random disturbance. We want to know if the constants are all the same.

Tukey multiple comparison Test

After performing ANOVA, Tukey HSD (honestly significant difference) post hoc test is generally used to calculate differences between group means as

where,

$$q = \frac{\bar{X}_1 - \bar{X}_2}{SE}$$

$$SE = \sqrt{\frac{S^2}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

S^2 is the error mean square from the analysis of variance and n_1 and n_2 are number of data in group 1 and 2 respectively.

Level of significance "p" is the probability signifies level of significance. The mentioned p in the text indicates the following:

$p > 0.05$	Not significant (ns)
$p < 0.05$	Just significant (*)
$p < 0.01$	Moderate significant (**)
$p < 0.001$	Highly significant (***)