"COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECT OF INDOCYANINE GREEN AND METHYLENE BLUE DYE AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN PATIENTS WITH MODERATE PERIODONTITIS - A RANDOMIZED CONTROLLED TRIAL"

BY

REG NO. IK0222002

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BELAGAVI, KARNATAKA

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This is to certify that the dissertation **"Comparative Evaluation of Antimicrobial Effect of Indocyanine Green and Methylene Blue Dye as Photosensitizers for Photodynamic Therapy as an Adjunct to Scaling and Root Planing in Patients with Moderate Periodontitis - A Randomized Controlled Trial"** *is a bonafide research work done by* ***REG NO. IK0222002.***

Dr. MENAKA K. B *M.D.S*
Professor and Head,
Department of Periodontics,
KAHER's KLE V. K. Institute of
Dental Sciences, Belagavi

Date: 18/4/25
Place: Belagavi

Professor and Head
Department of Periodontics
KLE V. K. Institute of Dental Sciences
Belagavi

Dr. ALKA KALE *M.D.S, PhD*
Principal,
KAHER's KLE V. K. Institute of
Dental Sciences, Belagavi'

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.

Date: 17/4/25
Place: Belagavi

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REG NO. IK0222002

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LIST OF ABBREVIATIONS

A.a	***Aggregatibacter actinomycetemcomitans***
ADPI	**Antimicrobial Photodynamic Inactivation**
ANOVA	**Analysis of variance**
aPDT	**Antibacterial Photodynamic therapy**
AsGaAl	**Arsenium Gallium Aluminum**
BMSC	**Bone Marrow Stem Cell**
BOP	**Bleeding on Probing**
BSRC	**Basic Science Research Center**
CAL	**Clinical Attachment Loss**
CFU	**Colony forming unit**
CHX	**Chlorhexidine**
CUR	**Curcumin**
FMBS	**Full Mouth Bleeding Score**
GI	**Gingival index**
ICG	**Indocyanine green**
LDD	**Local Drug Delivery**
LLLT	**Low Level Laser Therapy**
MB	**Methylene Blue**
MD	**Mechanical Debridement**
nm	**Nanometer**
NSPT	**Non-surgical Periodontal Therapy**
P.g	***Porphyromonas gingivalis***

P.i	***Prevotella intermedia***
P.n	***Prevotella Nigrescens***
PDLC	**Periodontal Ligament Stem Cell**
PPD	**Pocket Probing Depth**
PS	**Photosensitizer**
PTT	**Photothermal therapy**
RAL	**Relative attachment level**
RCT	**Randomized Controlled Trial**
ROS	**Reactive Oxygen Species**
S.m	***Streptococcus mutans***
SBI	**Sulcus Bleeding Index**
SFFR	**Sulcus Fluid Flow Rate**
SRP	**Scaling and Root Planing**
W	**Watt**
µm	**Micrometer**

ABSTRACT

INTRODUCTION

In recent years, photodynamic therapy (PDT) with the help of lasers has been used to decontaminate the pocket environment as it is a minimally invasive treatment that activates the immune system, has low systemic toxicity, and possesses high bactericidal properties. Combining lasers and photosensitizing agents can result in photo destruction of target cells due to cytotoxic effect in the presence of oxygen. The photokilling rate is highly related to interaction of reactive oxygen species produced, ability of photosensitizers in incorporating into microorganisms and light devices/microorganism type.

While methylene blue (MB) and indocyanine green (ICG) are present in the dental practice as photosensitizers there is an acute paucity of literature comparing their efficacy in terms of improvement of clinical parameters and suppression of periodontal pathogens.

AIM

To assess and compare the antimicrobial effects of indocyanine green and methylene blue dye as photosensitizers for photodynamic therapy as an adjunct to scaling and root planing in patients with moderate periodontitis.

MATERIALS AND METHODS

Sample size of 63 participants using standard formula was calculated. Participants were allocated into 3 groups as group I: SRP, group II: SRP + PDT with 0.5% ICG and group III: SRP + PDT with 1% MB.

Gingival index, Pocket probing depth, Clinical attachment loss and Number of colony forming units per ml were evaluated at baseline, 1 week and 1 month follow up periods. The statistical test to be done for the following parameters were: Descriptive statistics, Normality of data assessed by Shapiro – Wilk test, Kruskal Wallis ANOVA for intergroup comparison and Wilcoxon matched pairs test for intragroup comparison. Statistical significance to be accepted at a confidence level greater than 95% ($p < 0.05$).

RESULTS

The mean age group of the participants was 35.55 ± 7.79. Gingival index scores showed significant improvement between groups 1 and 2 and 1 and 3 from baseline to 1 week and 1 month interval (p=0.0001), however the differences were not significant between group 2 and 3 for the same and upon intragroup all the three groups showed statistically significant differences from baseline to 1 week and 1 month interval (p=0.0002). Pocket probing depths showed significant improvements amongst all the groups from baseline to 1 week and 1 month interval (p=0.0001), however between the group's comparison demonstrated significant differences only between group 1 and 2 and 2 and 3 at the follow-up periods (p=0.0003). The clinical attachment levels showed significant differences between the three groups from baseline to 1 week (p=0.0001), however the results were insignificant at 1 month interval. Upon intragroup comparison all the three groups showed significant improvement in CAL from baseline to week and 1-month intervals (p=0.0001).

The CFU/ml showed significant differences between the three groups from baseline to 1 week and 1 month interval (p=0.0001) and upon intragroup comparison the CFU/ml significantly reduced from baseline to 1 week and 1 month interval for all the three groups (p=0.0001).

CONCLUSION

Our investigation into the comparative antimicrobial efficacy of ICG and MB as photosensitizers for PDT yielded promising insights for combating moderate periodontitis as an adjunct to conventional ultrasonic scaling and root planing. Our findings suggest that the use of Indocyanine green mediated aPDT combined with 810 nm diode laser in contrast to conventional photosensitizers such as Methylene blue which have provided only moderate benefits as an adjunct acts as a potent antimicrobial agent, facilitating prolonged antimicrobial activity and offers a non-invasive and targeted approach to bacterial eradication, harnessing the oxidative power of light-activated photosensitizers to disrupt bacterial membranes and cellular structures.

KEYWORDS: Indocyanine green, Methylene blue, Laser, Periodontitis, Antimicrobial Photodynamic therapy.

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INTRODUCTION

"All truths are easy to understand once they are discovered; the point is to discover them"

- Galileo Galilei

Periodontal pathogens building a dental biofilm over the surface of teeth and the host immune response interact to cause chronic periodontitis, a common multifactorial condition, inflammatory in nature, disrupting the supporting attachment apparatus of the dentition. A major tenet of exfoliation of teeth in vulnerable patients, which can be fatal if treatment is not received, is attributed to periodontitis. Throughout the management of periodontitis, clinicians primarily seek to eradicate or lessen the count of burden of pathogens by eliminating soft and hard deposits over the tooth using manual instruments or an ultrasonic equipment as a part of the standard therapy for periodontitis. The goal of this approach is to eradicate periodontal bacteria in order to prevent the progression of inflammation and ongoing attachment loss.[1]

Non-surgical periodontal therapy typically results in notable clinical benefits. However, because of structural variances such as complex furcas, complexities over surfaces of the teeth along with infection engulfing the attachment apparatus of the teeth, nonsurgical periodontal therapy cannot completely eradicate periodontal pathogens on its own. As a result, the post-treatment periodontal healing process may be impacted by the residual bacterial reservoir.[2]

Supplementary systemic and local antimicrobial agents along with manual decontamination has facilitated bacterial reduction, particularly for cases unresponsive to conventional therapy.

Among its numerous disadvantages is its incapacity to effectively reduce the burden of periodontopathic bacteria, which includes the integration of inadequate amounts of medication in the sulcular fluid, development of resistant strains of bacteria brought about by their frequent use. Increased resistance to the majority of antibiotics used in periodontology, a rise in the number of patients with weakened immune systems and the fact that many different pathogens cause periodontal infections and require different antibiotics with varying risks of adverse reactions are all contributing factors to future challenges with antibiotic therapy. Therefore, alternate antibacterial methods for treating periodontal disease must be developed.[3]

To navigate beyond the risks and challenges that come from administering systemic antibiotics, other therapies such as local drug delivery (LDD) and anti-microbial photodynamic therapy (aPDT) have been employed. An oxygen-dependent treatment approach known as aPDT involves triggering a photosensitizer (PS) using light, thereby producing compounds cytotoxic to microorganism such as singlet oxygen and reactive oxygen species (ROS). Three nontoxic components are essentially used in photodynamic treatment (PDT), i.e. oxygen, nontoxic photosensitizer, and visible light. The target cells are bound by the photosensitizer (photoactivatable material), which activates them when light is applied. The capacity of aPDT to concentrate on cells specifically, the fact that it only activates when subjected to light, and the possibility that resilient strains of bacteria won't emerge are its key advantages.[4]

In patients with periodontitis, it serves as an immunomodulator, improves clinical parameters and demonstrates antibacterial activity against periodontal pathogens. PDT was initially used in 1903 to treat tuberculosis, and in 1975 it was found to be useful in treating skin cancer.

The use of this technique has been extensively studied in dentistry, including microbial infections and malignant lesions of the oral cavity. It being minimally invasive technique with high target specificity, the unlikeliness for it to develop bacterial resistance following multiple application it proves to be advantageous for combating periodontitis.[5]

PDT employs a number of photoactive compounds. A non-toxic photosensitizer that activates when exposed to light is ideal. The PS should have a number of ideal properties, including highly soluble, minimal cytotoxicity, rapid eradication, peak absorbance, an ideal proportion of fluorescent quanta to interchange quanta, a substantial output for oxygen singlet production, and storage and light stability. Additionally, the photosensitizer employed to treat periodontal infections ought to adhere to plaque and bacteria without generating any undesirable side effects, particularly undesired soft tissue discoloration. Additionally, it should be easy to access infections found in deeper periodontal pockets and should be acceptable to both patients and clinician. Despite the widespread usage of PDT dyes like MB and toluidine blue, green photosensitizers like ICG are now prevalent.[6]

MB is a member of the class of chemicals known as phenothiazinium and has been utilized widely for the photooxidation of both synthetic and natural compounds. With MB, two main photochemical pathways are typically seen.

Type I is the semi-reduced radical that is created when reduction factors transfer one electron to the MB triplet, and Type II is the singlet oxygen that is created when the resulting triplet potential passes on to oxygen. The high absorption band in the 650 to 900 nm range is what gives MB its distinctive colour.

When activated by an adequate wavelength, it can emit harmful oxygen free radicals with a radius of 0.20 μm.[7]

Through its photodynamics and primarily photothermal effect (PTT) at wavelength of 810 nm by a diode laser, ICG has an impact on the target tissue or cell. When ICG is used as a photosensitizer, PTT can cause cell damage by raising the intracellular temperature. This photosensitizer converts the majority of the light energy it absorbs into heat. ICG appears to have a mechanism of action that is not specific to any one species of bacteria and achieves its antibacterial action as a result of oxidative stress. It also shows minimum cytotoxicity to tissues of the host that are not the target. ICG has several benefits, such as minimal toxicity, no side effects, ease of use for both the patient and the practitioner, an absorption peak near the emission maximum of the dental diode lasers that are currently available in the market (about 800 nm), and quick elimination.[8]

The potential for different reactions to dye photosensitivity when one varies the intensity of light, wavelength, the amount of or lack of oxygen in the environment, the molecular makeup of the substances used as PS, ROS generated, the capacity of photosensitizers (PS) to integrate into microorganisms, and the type of light device or microorganism are all strongly correlated with the photokilling rate in antimicrobial photodynamic therapy.[9]

Although MB and ICG are used as PS in dentistry, there is an acute paucity of literature assessing how effectively they serve to enhance clinical parameters and inhibit periodontopathic bacteria. In order to juxtapose the antimicrobial properties of photosensitizers, i.e. indocyanine green and methylene blue for treatment with photodynamic therapy as a supplement to SRP amongst cases diagnosed with moderate periodontal disease, the current experiment was conducted.

AIMS AND OBJECTIVES

AIM:

In patients with moderate periodontitis, to evaluate and contrast antimicrobial properties of methylene blue dye and indocyanine green dye as photosensitizers for photodynamic therapy, which serves as a supplement to scaling and root planing.

OBJECTIVES:

- To assess the antimicrobial effectiveness using 0.5% Indocyanine Green dye in photodynamic therapy for patients with moderate periodontitis as a supplement to scaling and root planing.
- To assess the antimicrobial effectiveness using 1% Methylene blue dye in photodynamic therapy for patients with moderate periodontitis as a supplement to scaling and root planing.
- To collate antimicrobial efficacy of 0.5% Indocyanine green dye and 1% Methylene blue dye as photosensitizers in photodynamic therapy for patients with moderate periodontitis.

REVIEW OF LITERATURE

Antimicrobial Photodynamic Therapy (aPDT)

M Raghavendra et al. (2009)[10] explored the function of aPDT comprehensively, noting that the polysaccharides found in the oral biofilm's extracellular matrix are vulnerable to photodamage and extremely sensitive to singlet oxygen. PDT can work against bacteria that are resistant to antibiotics. Conventional scaling and root planing (SRP) may be perfectly complemented by aPDT. Venous stagnation and decreased tissue oxygen consumption occur during inflammation. Anaerobic species may develop more readily as a result of this pH shift and drop in oxygen levels. In these situations, PDT may lessen venous congestion in gingival tissues and enhance tissue blood flow in the microcirculatory system. Additionally, PDT may result in a 21–47% increase in gingival tissue oxygenation.

Dr. Dheeraj Khurana et al. (2014)[11] highlighted the benefits of aPDT as a simple clinical method in periodontics. Because PDT is a non-invasive local therapy, harm to the surrounding host tissues may be prevented and microflora at other places won't be disturbed when a sensitizer is applied. A light source is precisely supplied into the target area via a fiber optic connection. Both the patient and the operator benefit from PDT's rapid, comprehensive irrigation and pathogen removal in difficult-to-reach parts of the periodontal pocket. Following periodontal debridement, the chance of developing bacteremia can be reduced, beneficial for those with a medical history of "at risk." Antibiotic prescriptions are unnecessary, so the risk of adverse effects is eliminated. The area doesn't need to be anesthetized, and the bacteria are destroyed in less than 60 seconds.

It may destroy plaque biofilm, it makes it easier to access sites with restricted or deep access, it encourages osseointegration and avoid peri-implantitis in dental implantology.

Roopali Tapashetti et al. (2020)[12] claimed that a possible new therapeutic strategy for eliminating harmful bacteria in periodontal and peri-implant illnesses is photodynamic therapy. Three nontoxic components are essentially used in photodynamic therapy: oxygen, a nontoxic PS, and visible, harmless light. Chemicals known as PSs, when stimulated through light of correct wavelength, lead to photodynamic treatment. When it is activated, leads to the generation of ROS and singlet oxygen causing certain cells to become cytotoxic.

Methylene blue as a photosensitizer for aPDT

Rosangela de Carvalho Goulart et al. (2010)[13] assessed the effectiveness of PDT using MB and erythrosine against *A. actinomycetemcomitans* and it was found that erythrosine was more effective than MB at killing *A. actinomycetemcomitans* bacterial cells in planktonic (75%) and biofilm (77%) cultures in contrast to 50% and 54%, respectively with MB. The study concluded that PDT using PS such as erythrosine or MB could be a reasonable solution for the decontamination of periodontal pocket associated with periodontal disease of the aggressive form.

J. Lui et al. (2011)[14] executed an interim clinical investigation in which 24 non-smokers diagnosed with CGP administered aPDT using 1% MB solution and LLLT supplementing NSPT whereby the test teeth outperformed the control teeth with respect to PPD at one month, BOP with a substantial drop in sulcular fluid quantity amongst the groups at one week and a further drop at one month in the test group.

At one week, IL-1b levels in SF were lesser across the experimental sites in contrast to control sites and at three months, with negligible appreciable changes in the assessed parameters amongst them, indicating that an interim application of LLLT and aPDT may be a useful addition to NSPT for cases diagnosed with CGP.

Gisele N. Campos et al. (2012)[15] demonstrated aPDT in single-rooted teeth associated with residual periodontal pockets as a supplement to SRP using a portable diode laser with settings 60 mW, 129 J/cm^2 and 660 nm and MB as a PS (10 mg/ml). At three months, the PDT + SRP group showed greater drop with respect to PPD as well as gain with CAL whereas clinical metrics improved considerably following both therapies ($p<0.05$). PDT may be an alternate treatment approach in supportive periodontal maintenance, and it has shown substantial clinical advantages for persistent pockets in single-rooted teeth when used as an adjuvant to mechanical debridement.

Mohammad Berakdar et al. (2012)[16] investigated the additive effectiveness of aPDT over SRP in long-term clinical research involving 22 patients with chronic periodontal disease. Two SRP-only and two SRP-plus-aPDT treated teeth were considered in each patient. The proportion of teeth that tested for the presence of BOP decreased following both forms of therapy.

The CAL measured 8.1 ± 1.3 mm with combination therapy in contrast to 7.2 ± 1.2 mm with only SRP and improvement was noted at one, three, and six months following both forms of therapy. After six months, the PPD improved to 2.9 ± 0.8 mm with combination therapy than 2.4 ± 0.6 mm with only SRP at baseline. At six-month interval the higher PPD reduction attained by combination therapy was significant statistically.

Maybel Lages Balata et al. (2013)[17] described aPDT as a supplement to SRP amongst 22 CGP cases. Subgingival irrigation using 0.005% MB dye was the first stage in PDT, which was only done on one side of the mouth. The arsenium-gallium-aluminium low power laser (power of 100 mW, wavelength of 660 nm, energy of 9 J with 90 seconds per site, using a 600 μm diameter tip) was applied two minutes after the PS was applied. Following therapy, both groups showed improvements in clinical parameters however the difference between them was insignificant. Post six-month interval, the test group's PPD dropped to 2.83±0.47 mm from 5.11±0.56 mm in contrast to control cases and CAL improved to 3.41±0.84 mm from 5.49±0.76 mm with respect to experimental cases than the control cases indicating that aPDT barely offered additional benefits to SRP alone.

Véronique S. Müller Campanile et al. (2013)[18] evaluated the local biological, microbiological, and clinical effects of PDT, administered once or twice over a one-week period following ultrasonic instrumentation, in residual periodontal pockets in 28 individuals in good overall health. A blunt irrigator tip was used to apply MB to the pockets and the dye was activated using laser irradiation at wavelength 670 nm. Single and double application of PDT (2.9± 1.1 mm and 2.8±1.1 mm respectively) had significantly lower PPD at month three than only SRP (3.5±1.2 mm).

The microbial identification rates depicted no drastic variations for any group. Serum amyloid A, α-2 macroglobulin fibrinogen, C-reactive protein and procalcitonin showed a substantial overall decrease from baseline to month six, suggesting that 1 or 2 applications of aPDT offered further advantages versus SRP only.

Letícia Heineck Alvarenga et al. (2014)[19] assessed the antibacterial properties of PDT with MB against biofilm-organized *A. actinomycetemcomitans* in vitro. Bacterial biofilm could not be rendered inactive by MB or red laser alone. When compared to the control group, the antimicrobial photodynamic inactivation (ADPI) group had variations that were contingent on the duration of exposure. At one and three minutes of irradiation, the differences were not significant statistically across the APDI groups. However, 99.85% of the bacteria were reduced after 5 minutes of APDI ($p = 0.0004$). Furthermore, after 5 minutes of APDI, the biofilm deteriorated its structure.

SJ Pulikkotil et al. (2016)[20] evaluated the effectiveness of PDT in lowering *A. actinomycetemcomitans* in 20 individuals with periodontitis through a RCT. BOP only considerably decreased across the test sites at 3 months, although the improvement in clinical parameters was significant at both 1- and 3-month interval from baseline in contrast to NSPT. Nonetheless, a discernible variation was not noted in the test sites in terms of *A. actinomycetemcomitans* quantification. According to the study's limitations, PDT used as an adjunct to SRP does not quantitatively lower *A. actinomycetemcomitans*.

Mansour et al. (2017)[21] compared manual debridement for treating periodontal inflammation in 70 patients with prediabetes, both with and without the adjunct aPDT. A blunt needle was used to apply MB (0.005%) in the periodontal pocket followed by irradiation using diode laser of setting 150mW, 670 nm, over 60 seconds using a flexible tip). In groups 1 and 2, baseline was substantially greater than the 3-month follow-up in terms of PI, BOP and PPD. However, the difference in clinical parameters at 3- and 6-month intervals were not significant statistically and comparable to that at baseline.

Crestal bone loss and HbA1c levels did not depict drastic alterations amongst test as well as control sites at 3- and 6-month follow-up. The investigation came to the conclusion that adjunct aPDT makes a negligible effect in this area.

Letícia Helena Theodoro et al. (2018)[22] examined the efficacy of NSPT on 51 smokers with chronic periodontitis. These treatments included 500 mg amoxicillin with 400 mg systemic metronidazole and numerous adjunctive applications of aPDT with 1 ml of MB at 10 mg/ml. SRP was carried out for every subject. Patients administered with both systemic antibiotics and aPDT demonstrated greater gain in CAL, reduced BOP, and considerably lower mean PPD after 180 days. The levels of *Prevotella nigrescens* and *P. gingivalis* after 180 days, decreased considerably in the antibiotic group while only P. *nigrescens* levels decreased in the aPDT group with respect to moderate pockets. In terms of deep pockets, *Prevotella intermedia, P. gingivalis* and *P. nigrescens* reduced drastically after 180 days, while group aPDT showed a decrease in levels of *P. nigrescens* and *P. intermedia*. Antibiotics along with aPDT therapies considerably enhanced the efficacy of SRP in smokers with periodontitis.

Naira Maria Rebelatto Bechara Anderea et al. (2018)[23] assessed supplementation of SRP, with locally administered clarithromycin in combination with aPDT utilizing MB 10 mg/ml amongst 72 patients with periodontal pockets associated with single-rooted teeth. In comparison to SRP alone its combination with antibiotics and aPDT expressed lesser PPD at three months. At six months, however, the antibiotic groups experienced a larger decrease in mean probing depth than SRP or aPDT. Only the combination of SRP, antibiotic and aPDT demonstrated a drastic increase in CAL. Therefore, when used in conjunction with clarithromycin, ultrasonic periodontal debridement offers more clinical benefits than when used in conjunction with aPDT.

Arash Azizi et al. (2019)[24] evaluated the efficacy of PDT over *Streptococcus mutans* through an in vitro experimental investigation utilizing curcumin (CUR) and 0.02% MB PSs. The proliferation of bacterial colonies of *S. mutans* was significantly inhibited by chlorhexidine and combination of curcumin and LLLT (460 nm). The experiment concluded that *S. mutans* colonies can be effectively eliminated by PDT mediated by MB and CUR.

Nahid Derikvand et al. (2020)[25] assessed how 50 patients' clinical periodontal parameters were impacted by aPDT (wavelength = 660 nm, power = 150 mW) irradiation with MB as a supplement to SRP. At baseline, barely discernible alterations with respect to clinical information amongst individuals was marked. When compared to the baseline, PI, Gingival index (GI), and PD all showed significant improvement ($p<0.05$). Because aPDT can enhance therapeutic outcomes without causing any negative side effects, in addition to SRP, it can be considered a sound and effective measure.

Mohammed N. Alasqah (2024)[26] in his comprehensive review and analysis to appraise the impact of MB-mediated adjunctive aPDT on periodontal diagnostic imaging and clinical outcomes amongst cases diagnosed of periodontitis in contrast to manual debridement alone. When MB-mediated aPDT was applied instead of MD alone, the meta-analysis showed statistically greater enhancement in PI, PPD, and BOP scores, however that was not the case with respect to CAL between the test and control groups at the final follow-up visit. A total of 11 trials were a part of the review. According to the review's findings, patients with periodontitis benefit from improved periodontal clinical outcomes, including as PI, PD, and BOP, when MB-mediated aPDT is administered in addition to traditional MD.

Indocyanine green as a PS for aPDT

Kura Srikanth et al. (2015)[27] examined the effects of 5 milligrams per millilitres ICG irradiated using 810 nm soft tissue laser as a supplement to NSPT amongst 30 individuals diagnosed with chronic periodontitis on the proportion of live bacteria and host tissue damage in. The percentage of live bacteria in sites receiving laser and ICG at 1 week interval was drastically lesser than the control sites. Lactate dehydrogenase levels show that ICG treatment does not appear to damage tissue. At the conclusion of the trial period, CAL and PD comparisons showed no discernible differences between areas treated with laser and ICG. According to the study's findings, laser-activated ICG dye can be utilized as a supplement to nonsurgical periodontal therapy and may increase the potential advantages of SRP.

Abbas Monzavi et al. (2016)[28] assessed the effectiveness of ICG mediated aPDT additional to SRP only to treat 50 participants diagnosed with chronic periodontitis. ICG was irradiated with a diode laser at a power of 200 mW and wavelength of 810 nm. There were appreciable differences in terms of BOP, FMBS and PPD however these differences were not reciprocated by CAL, PI and FMPS. According to the study's findings, using aPDT as an adjuvant method led to a considerable reduction in PPD and total remission of inflammation. But in terms of CAL gain and PI, aPDT had no additive advantages.

Saurabh H Shingnapurkar et al. (2016)[29] analysed supplemental aPDT on chronic periodontitis utilizing an ICG irradiated with 810 nm diode laser. There were no alterations in the clinical parameters at baseline for the 2 groups. However, there was a drastic change appreciated in PPD and RAL at 3 months between the two groups.

The study found that in chronic periodontitis patients, supplementary PDT can improve the clinical results of conventional SRP.

Kasra Karamifar et al. (2016)[30] investigated the impact on *P. gingivalis* using ICG (Emundo solution) based photodynamic therapy at settings 300 mW, 11.5 J/cm2 and 810 nm in contrast to 2% metronidazole and chlorhexidine gel. All the 3 groups showed a drop in the colony forming units after 24 and 48 hours and the inhibitory zone radius decreased. It was determined that, within the constraints of this in vitro investigation, 2% metronidazole outperformed PDT with Emundo in terms of bacterial reduction, even if the latter greatly decreased the number of *P. gingivalis*. Additionally, 2% CHX gel had a noticeably stronger antibacterial effect on *P. gingivalis* than PDT with Emundo.

Kunal S Sethi et al. (2017)[31] compared and assessed results amongst 30 patients with chronic periodontitis treated with PDT utilizing ICG. All clinical measures including SBI, CAL, PPD, PI and Gingival recession showed a significant decline at test sites. In juxtaposition to control samples, the experimental sample's anaerobic culture of plaque samples revealed a significant drop in bacteria. The study concluded that when used in conjunction with SRP in PDT to treat chronic periodontitis, ICG can serve as an alternative to other PSs.

Chetan Purushottam Raut et al. (2018)[32] intended to compare and assess the results of PDT utilizing ICG in treating 50 patients with SRP who had chronic periodontitis. After six months, there was a drastic decrease in CAL, BOP along with PPD across the experimental in contrast to control sites. Nevertheless, results of PI intergroup comparison were not appreciable. The anoxic culture of plaque samples at the test site similarly showed a considerable decrease in bacteria in contrast with the control sites.

The authors found indocyanine green-mediated PTT to better serve as an adjuvant to SRP and a substitute for aPDT.

Nasir Zeeshan Bashir et al. (2020)[33] in his comprehensive review described clinical results from seven eligible studies and meta-analyses were collected and coupled, expressed as Mean ±SD. The mean extra reduction for PPD brought on by adjunctive ICG-PDT was 1.17 as well as 1.06 milli meters at 3 as well as 6-month interval correspondingly. At three as well as six months, the mean additional gain for CAL with supplemental ICG-PDT was 0.70 mm and 1.03 mm, respectively. No research found any negative effects. Better treatment outcomes were obtained when ICG-PDT was used as an adjuvant in NSPT at three- and six-months following therapy.

Chun-Pin Chiang et al. (2019)[34] investigated the effectiveness of ICG based aPDT amongst 35 test and 30 control teeth across 22 individuals with periodontal pockets resistant to manual debridement. The vitality of BMSCs and PDLCs was restored on day 4 in ICG-treated cultures, along with suppression of their metabolic activity. PPD, CAL, BOP, and plaque scores were all slightly lower in all analyzed teeth at T1 and T2, whereas IL-1 band MMP-8 was significantly lower at T2. At T1, the PTT group experienced slightly larger decreases in PPD and CAL, a more noticeable decrease in BOP, and a much lower IL-1 band MMP-8 than the controls. According to the study's findings, PTT based on ICG-diode lasers is compatible with the periodontium and aids in the faster reduction of inflammation in the gingival region in periodontal pockets that are difficult to debride mechanically.

Kaveri Kranti Gandhi et al. (2019)[35] assessed the effectiveness of PDT and LLLT in treating chronic periodontitis as a supplement to SRP. At all subsequent appointments (1,3,6, and 9 months), NSPT for chronic periodontitis that combined LLLT and PDT with SRP was noticeably more successful than SRP solely in lowering CAL, PPD, GI, and *P.gingivalis* and *A. actinomycetemcomitans* levels.

Greta Hill et al. (2019)[36] evaluated in a prospective clinical investigation, the effectiveness of aPDT using 0.1 mg/ml ICG irradiated with a diode laser at a power of 100 mW and wavelength of 808 nm amongst twenty CGP cases. No assessed measures showed any appreciable variations between the control and test groups on Day 0. The median values for PPD, RAL and BOP significantly decreased in both groups following three months of treatment. Two weeks after treatment, the experimental group exhibited noticeably lower mean values for the SFFR.

Within the study's parameters, the only noticeable alteration amongst the aPDT samples and the control samples was a transient drop in SFFR in the latter group during the first follow-up. This study is optimistic because no adverse effects were noted, but it does not conclusively confirm ICG-based aPDT under the circumstances that were employed.

Karuna Joshi et al. (2020)[37] ascertained the potency of ICG based aPDT, auxiliary to SRP across 29 CGP cases. It was observed that both groups' modified SBI and PI considerably decreased at 3 months interval, despite the fact that the intergroup comparison for both measures yielded an unimportant result which was in contrast for parameters such as CAL and PPD and CAL whereby significant results were observed at sites treated with ICG over 3-month time interval. Indocyanine green based aPDT as an adjuvant improved the potency of SRP.

Sartaz Rahman et al. (2020)[38] evaluated how effectively 1.2% gel of simvastatin with aPDT worked in addition to SRP in managing 33 samples amongst eleven chronic periodontitis patients. Three months following treatment, the levels of RANKL in GCF, DNA copy counts of *P. gingivalis*, and clinical parameter scores (PI, PBI, PPD, and RAL) of all three groups were significantly lower than baseline but, there were no drastic changes upon intergroup comparison. Juxtaposing aPDT with control sites, there was a slightly greater decline in RAL and PPD levels in the statin sites, as well as in the biochemical and microbiological parameters, however these differences were not statistically noteworthy. At three months, Numbers of *P. gingivalis* DNA copies, substantially correlated negatively with PBI as well as API scoring in case of only SRP, while they significantly correlated positively with PPD scores in the SMV group. According to the study, clinical, microbiological and biomolecular parameters can all be successfully decreased by SRP alone, aPDT, and 1.2% SMV local medication administration as an adjuvant to SRP.

MATERIALS AND METHODS

SOURCE OF DATA

The research was subjugated as a single-blinded, randomized controlled clinical experiment at the Dr. Prabhakar Kore Basic Science Research Centre (BSRC), KLE Academy of Higher Education and Research, Belagavi, Karnataka, and the Department of Periodontics, KLE Vishwanath Katti Institute of Dental Sciences, KAHER. Before undertaking the study, the Ethical Committee of KAHER's KLE Vishwanath Katti Institute of Dental Sciences, Belagavi, granted ethical clearance (Annexure 1). Under the Clinical Trials Registry-India (ICMR-NIMS), the randomized controlled single-blinded clinical trial was registered (CTRI Reg. No: CTRI/2024/09/073681).

For the study, 63 patients who presented at Outpatient Department of Periodontics at KAHER's KLE Vishwanath Katti Institute of Dental Sciences, Belagavi, and were identified with moderate chronic periodontitis (1999 International Workshop for Classification of Periodontal Disease and Condition) were opted for.

The study comprised patients who were in par with below mentioned inclusion and exclusion benchmark.

INCLUSION CRITERIA

- Adults over the age of eighteen who do not smoke
- Patients with minimum 20 teeth present
- Moderate chronic periodontitis that has not been treated with at least two single or multirooted teeth on respective sides of the mouth with a probing depth of at least 5 mm, interproximal loss of attachment of at least 3 millimeters and evidence of loss of bone radiographically.
- Informed consent granted

EXCLUSION CRITERIA

- Pregnant or lactating women.
- Systemic disease affecting periodontal treatment outcome.
- Use of immunosuppressive agents.
- Patients on antibodies or anti-inflammatory drugs in the past 3 months.
- Periodontal treatment in the past 6 months.
- Patients allergic to the use of dyes.
- Patients presenting with a history of ocular problems.

CLINICAL ARMAMENTARIUM

1. Surgical drape
2. Mouth mask
3. Disposable gloves
4. Explorer, Tweezers, Straight Probe, and Mouth Mirror
5. William's probe for periodontal disease
6. Ultrasonic scaling tips and an ultrasonic scaler unit
7. Kidney tray

8. Cotton swabs and gauze
9. 1% Methylene blue (Fischer Scientific)
10. 0.5% Indocyanine green (Aurogreen®, Aurolab, Madurai, Tamil Nadu)
11. Sterilized set of Gracey curettes

LABORATORY MATERIALS

1. Sterilized microcentrifuge tubes – Eppendorff tubes
2. Labotech bacteriological incubator
3. 9 ml dilution blanks
4. Sterile petri plates
5. Sterile 1 mm pipettes
6. Nutrient agar medium

LASER UNIT

IndiLase Soft tissue laser (660 nm, 810 nm)

- Peak power of 0.1 W for 60 seconds, 6 Joules per site
- Continuous mode using multifibre optic tip

PREPARATION OF METHYLENE BLUE SOLUTION

The photosensitizer utilized in this study, 1% methylene blue, was synthesized whereby 100 cc of distilled water was mixed with one gram of methylene blue, which had been weighed, to yield 1% methylene blue.

PREPARATION OF INDOCYANINE GREEN SOLUTION

The photosensitizer utilized in this work, 0.5% Indocyanine Green, was synthesized whereby 100 milliliters of distilled water was infused with 0.5 grams of indocyanine green, which had been weighed, to yield 0.5% indocyanine green.

STUDY DESIGN

Patients were branched into test as well as control groups through straightforward random sampling technique (computer-based software) as part of the randomized controlled single-blinded clinical experiment.

Under local anesthetic, patients in Group 1 (n = 21) underwent root planing and full mouth ultrasonic scaling.

Following scaling and root planing, the periodontal pockets in Group 2 (n = 21) were filled with a 0.5% Indocyanine Green solution and let to settle for three minutes. The excess was washed off carefully.

Following scaling and root planing, the periodontal pockets in Group 3 (n = 21) were filled with 1% Methylene Blue solution and let to settle for three minutes. The excess was washed off carefully.

The investigation comprised cases who signed an informed assent proforma.

To ensure a methodical and systematic collection of all the observations and data, a unique proforma (Annexure VI) was created for the study. The patient's biographical information, primary complaint, and previous dental history were all included in the unique proforma.

Following the recording of basic data, a mouth mirror, explorer, graduated William's periodontal probe, and tweezer were used to collect microbiological samples and perform a clinical examination in a dental chair under standard lighting circumstances. At baseline, one week, and one month following therapy, the clinical parameters were assessed.

Using the CEJ as a reference point, all parameters were measured on four different sites per tooth (mesial, distal, buccal, and lingual/palatal) using a William's periodontal probe.

The clinical parameters listed below were noted:

1. Gingival index
2. Probing pocket depth
3. Clinical attachment loss

Gingival Index (Loe and Silness, 1963)

The gingival index, which was first proposed in 1963, is used to evaluate the amount and severity of gingival inflammation in both broad population groups and individual patients. The four gingival regions of the tooth—the lingual, distal, mesial, and facial—were evaluated for inflammation using this procedure, and a score ranging from 0 to 3 was assigned. A periodontal probe was used to calibrate bleeding along the gingival crevice's soft tissue wall.

Score	Criteria
0	Absence of inflammation, normal gingiva
1	Mild inflammation, slight change in color, slight edema and no bleeding on probing
2	Moderate inflammation, redness, edema, glazing and bleeding on probing
3	Severe inflammation, marked redness and edema, ulcerations, tendency to spontaneous bleeding present

Calculation of the index

A tooth's gingival index was calculated by summing the scores from its four sections and dividing the total by four. The entirety of teeth inspected was divided by the sum of the indices for each tooth. This gives the individual's gingival index.

Scale for patient evaluation

Gingival scores	Condition
0.1-1.0	Mild gingivitis
1.1-2.0	Moderate gingivitis
2.1-3.0	Severe gingivitis

Probing Pocket Depth (PPD)

From the base of the pocket to the crest of the marginal gingiva, the depth of the probing pocket was acquired utilizing William's periodontal probe. To identify the areas of deepest penetration, the probe was placed correspondent to the vertical axis of the tooth and "walked" circumferentially across every surface. The investigation comprised pocket depths of at least 5 mm.

Clinical Attachment Loss (CAL)

The extent from the depth of the pocket and a stationary location over the crown, i.e. cementoenamel junction (CEJ), was used to calculate clinical attachment loss. CAL was estimated in the current investigation utilizing a William's periodontal probe, with CEJ serving as a reference point.

The CAL is equal to the pocket depth when the margin of the gingiva and the CEJ line up. The extent from the margin of the gingiva and the CEJ was added to the depth of the pocket because the loss of attachment is larger when the gingival margin is positioned apical to the CEJ.

PROCEDURE FOR PERIODONTAL THERAPY

Under local anesthetic, all patients had a complete mouth SRP procedure.

Group 2's periodontal pockets were flushed with 0.5% indocyanine green dye (Photosensitizer) after scaling and root planing were finished, and they were left for three minutes. The excess was washed off carefully.

Group 3's periodontal pockets were flushed with 1% methylene blue dye (Photosensitizer) after scaling and root planing were finished, and they were left for three minutes. The excess was washed off carefully.

Antimicrobial photodynamic therapy was performed in Group 2 and 3 at wavelengths 810 nm and 660 nm respectively with an InGaAs semiconductor diode laser at a power of 0.1 W (6 Joules) using a multifiber tip. The tip was placed at the gingival margin pointing towards the sulcus and laser irradiation was done for 60 seconds at 0.1W setting per site. After the irradiation, the site was cleaned using wet cotton rolls.

MICROBIOLOGICAL ASSESSMENT

For the experimental and control sites, baseline subgingival plaque samples were acquired from the location exhibiting the periodontal pocket of the greatest depth. After a week and a month, the same sites were re-sampled. Every spot that was chosen was separated and dried. A sterile Gracey's curette was then inserted in order to collect samples of subgingival plaque. Within 20 minutes after collection, the samples were taken in a sterile microcentrifuge tube filled with thioglycolate broth (Transport media) and delivered to KAHER's Dr. Prabhakar Kore Basic Research Center in Belagavi for microbiological analysis.

1. Sample Preparation and Serial Dilution

After preparing a sterile nutritional agar medium, it was transferred onto sterile petri dishes and let to set. The subgingival sample collected in the thioglycolate broth (Transport media) was mixed well by vortexing. The dilution of the sample was prepared using sterile saline solution in test tubes whereby 1 mL of the original sample was transferred into 9 mL of diluent (10^{-1} dilution) using a new sterile pipette.

2. The Plating Process

Spread Plate Method: Using a sterile swab, 0.1 ml of a suitable dilution was pipetted across a nutrient agar plate's surface and dispersed uniformly. Before incubation, the plate was given time to absorb the sample.

3. Incubation

For twenty-four hours, after being inverted, the plates had to incubate at 37°C.

4. Colony Calculation and Counting

A colony counter was used to count the colonies that were visible on the plates following incubation.

POST-OPERATIVE INSTRUCTIONS

Following the periodontal therapy procedure, each patient received standardized post-operative instructions. Every patient received advice on the correct tooth brushing technique consistently. Additionally, during the trial period, the patients were told not to use any mouthwashes or antibiotics.

STATISTICAL ANALYSIS

After entering the study data into Microsoft Excel, it was exported to IBM Statistics' Statistical Package for Social Sciences (SPSS) Version 25. The Shapiro-Wilk test was used to determine whether the data was normal. The scores of all parameters at different treatment time points in three groups did not follow a normal distribution (. *$p<0.05$). Therefore, the non-parametric tests were applied. The mean, standard deviation, and percentages of descriptive statistics were acquired. Using SPSS version 25, IBM Statistics, USA, a one-way ANOVA/Kruskal Wallis test and a post-hoc/Mann Whitney-U test analysis were performed for intragroup comparison. At a 95% confidence level ($p < 0.05$), statistical significance was considered.

Figure 1: Clinical Armamentarium

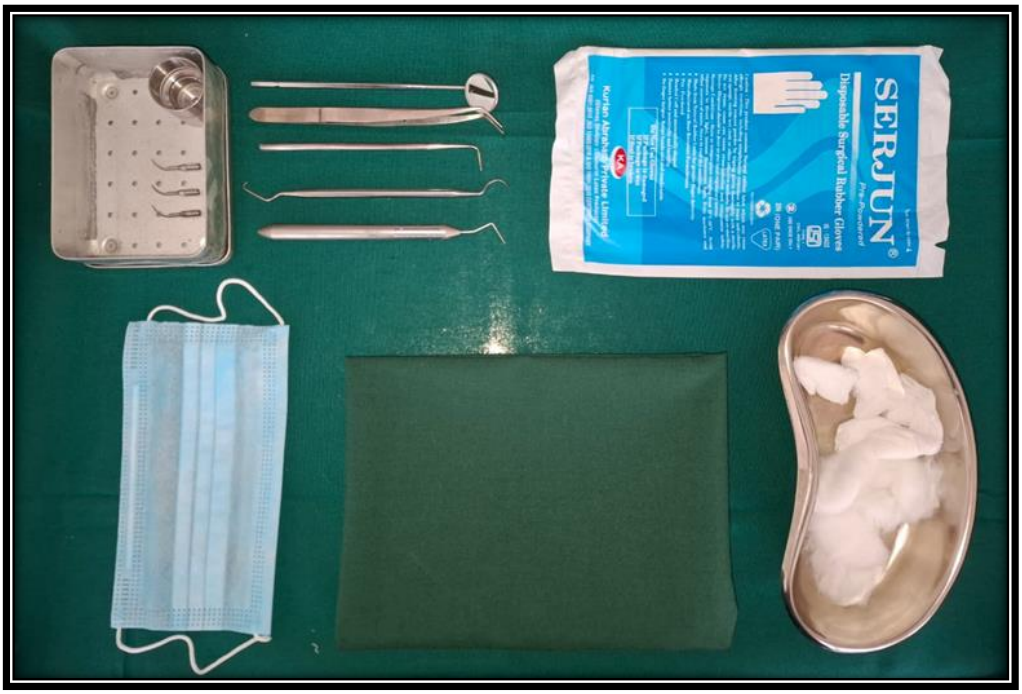


Figure 2: Sterilized Set of Gracey's Curettes and Microcentrifuge Tube for the Collection of Subgingival Plaque Samples

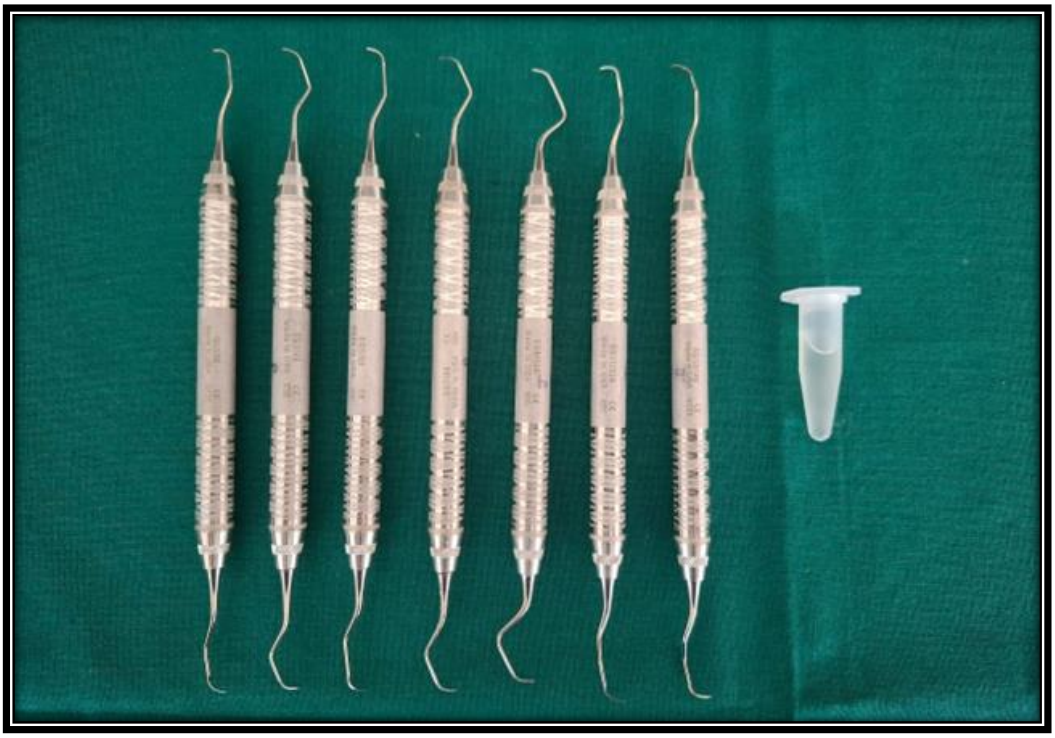


Figure 3: Methylene blue dye and Indocyanine green dye as photosensitizers for Photodynamic therapy

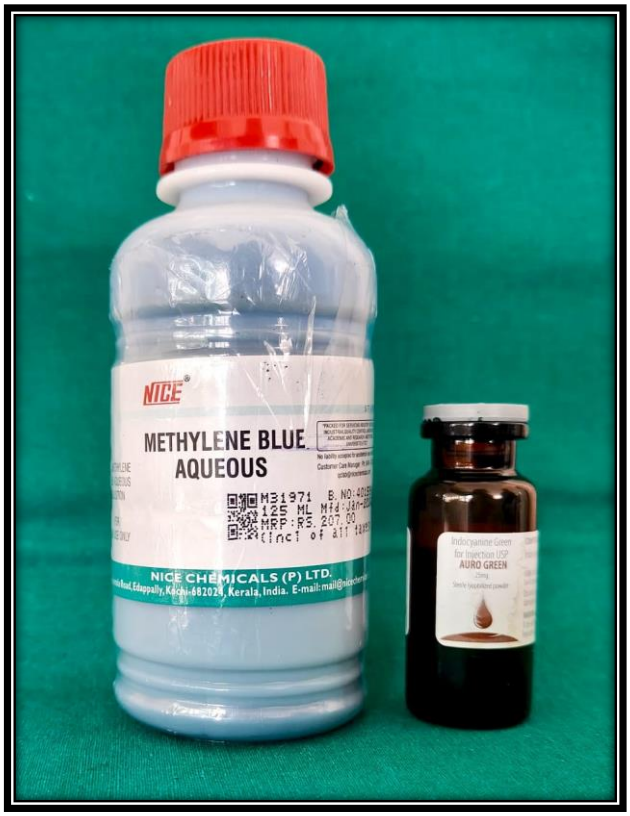


Figure 4: Indium-Gallium-Arsenide-Phosphorus (InGaAsP) Laser

Figure 5: Labotech Bacteriological Incubator

Figure 6: Laboratory Reagents

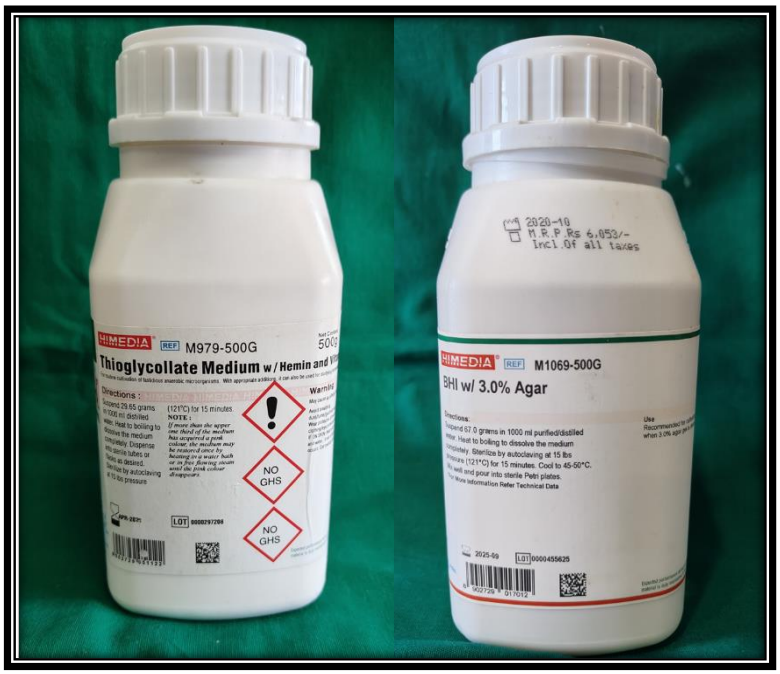


Figure 7: Measurement of Clinical Parameters Using William's Periodontal Probe at Baseline for Group 2

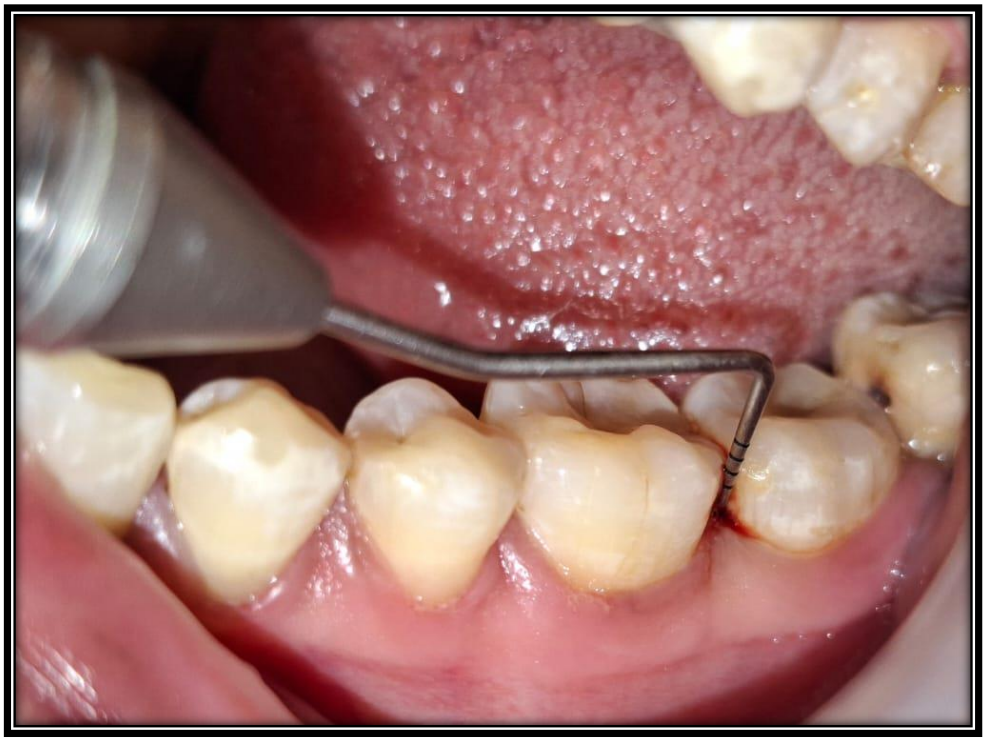


Figure 8: Measurement of Clinical Parameters Using William's Periodontal Probe at Baseline for Group 3

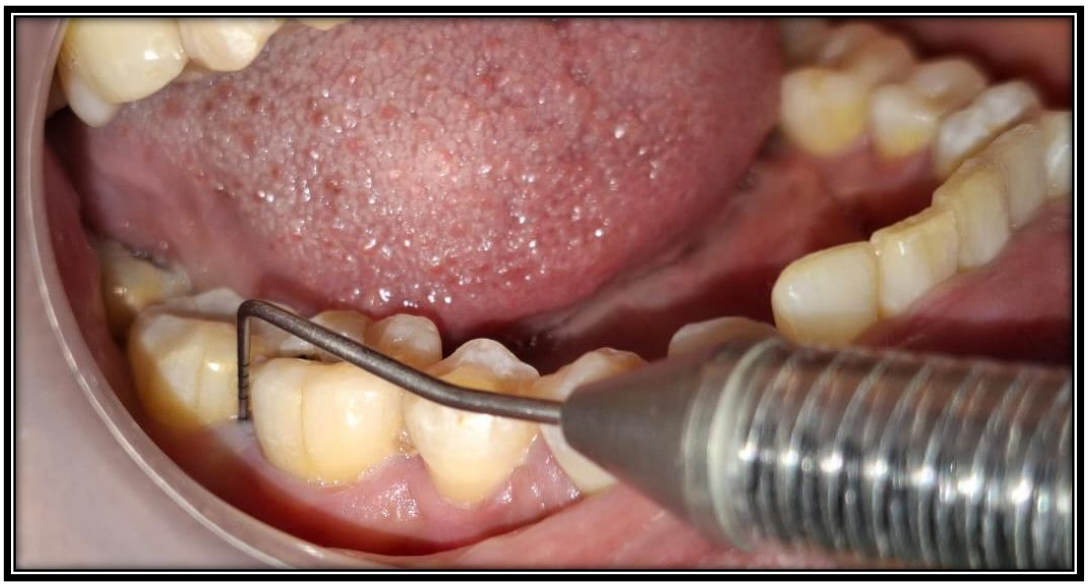


Figure 9: Collection of Subgingival Plaque Samples Using Sterilized Gracey's Curettes

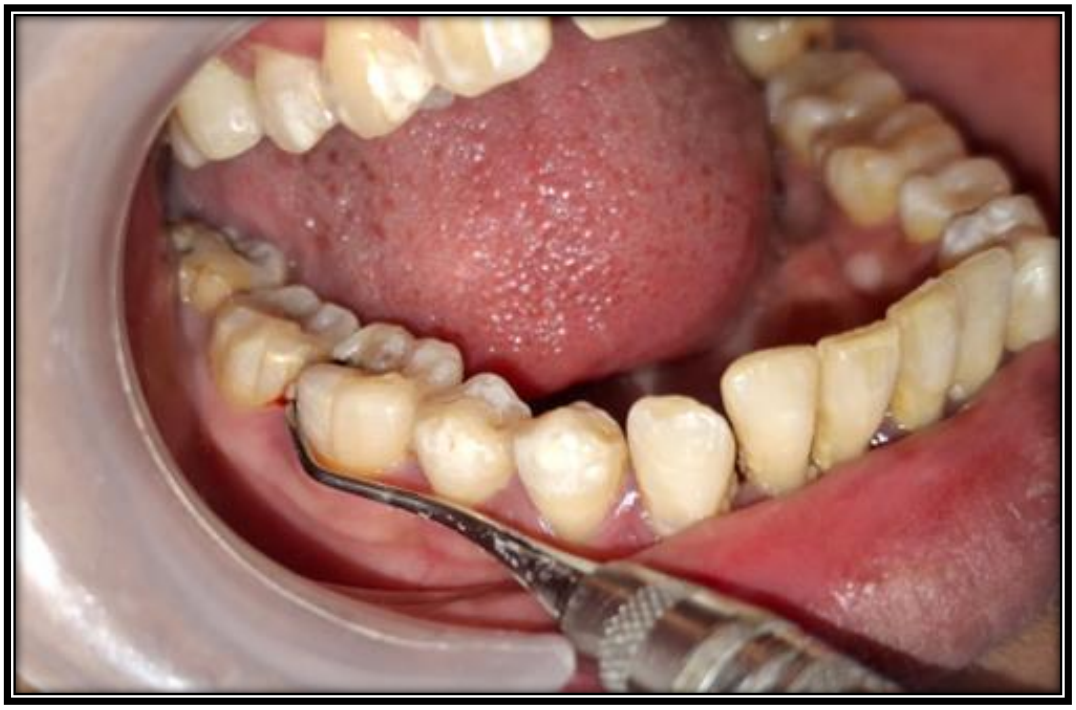


Figure 10: Application of Indocyanine Green Dye and Laser

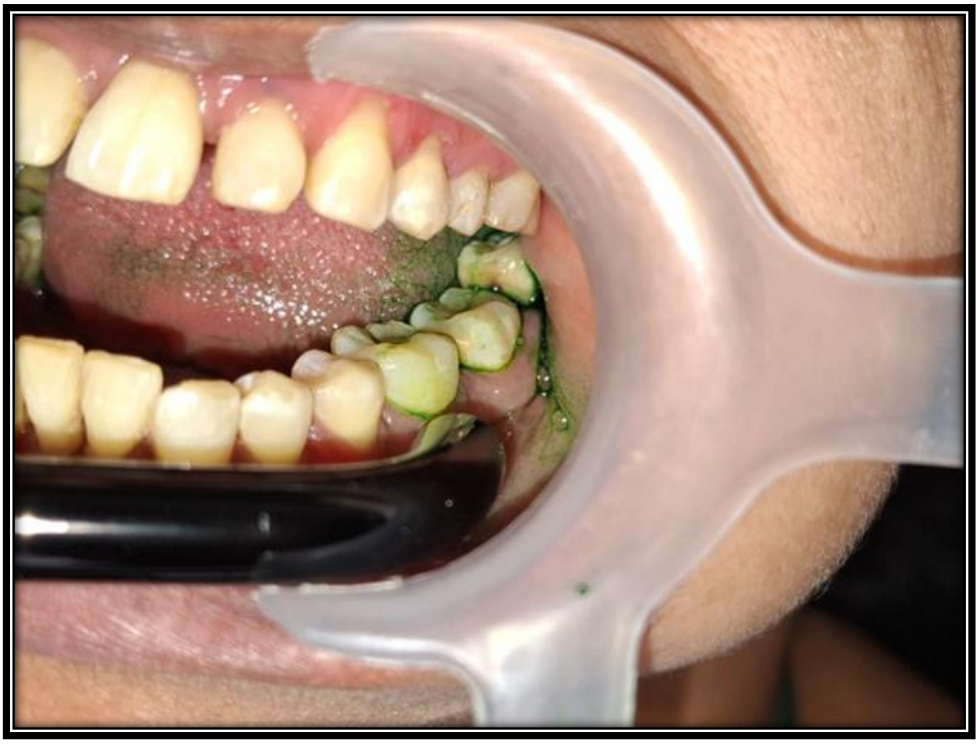


Figure 11: Application of Methylene Blue Dye and Laser

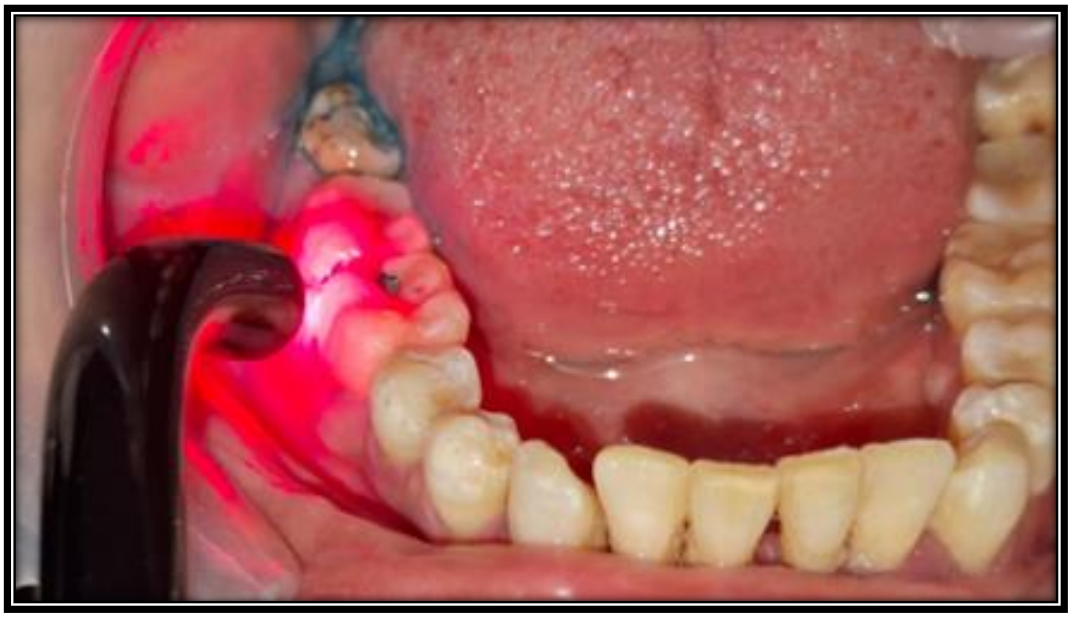


Figure 12: 1 Week Post-Operative for Group 2

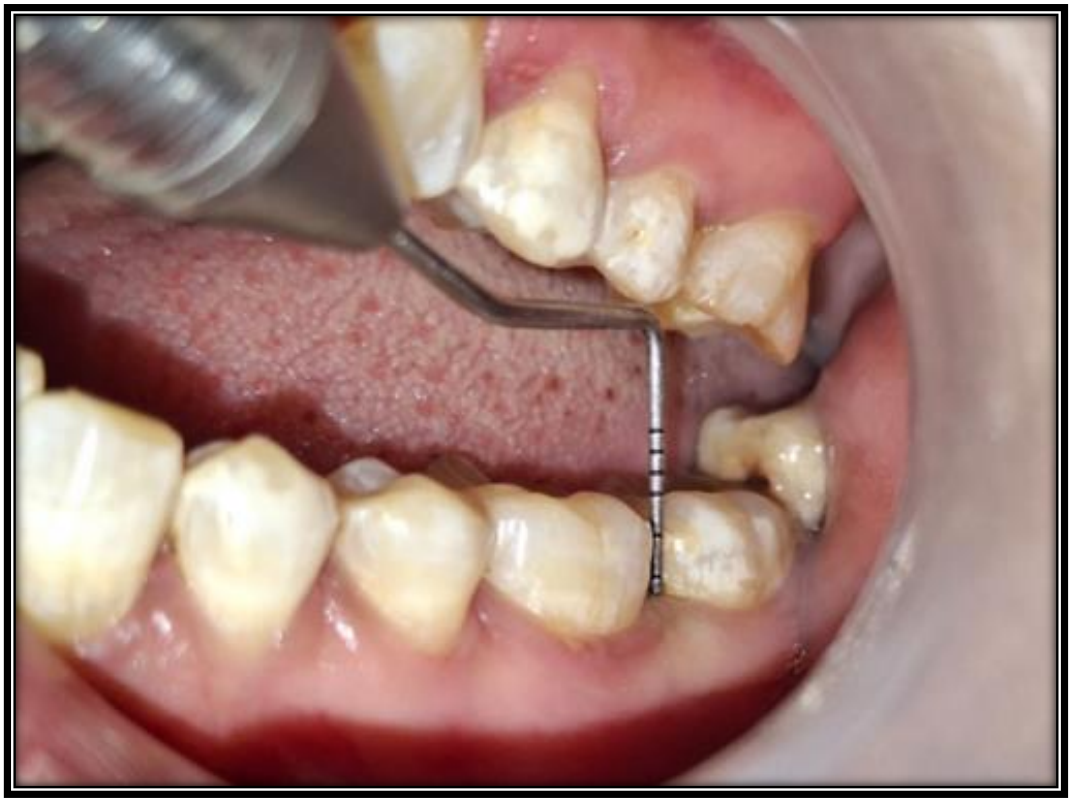


Figure 13: 1 Week Post-Operative for Group 3

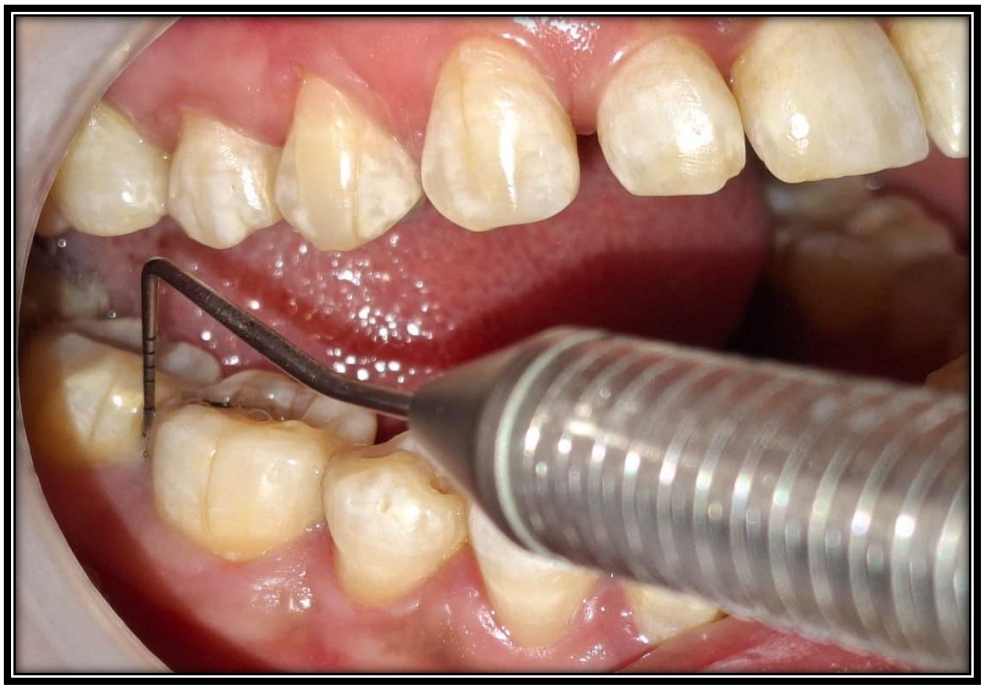


Figure 14: 1 Month Post-Operative for Group 2

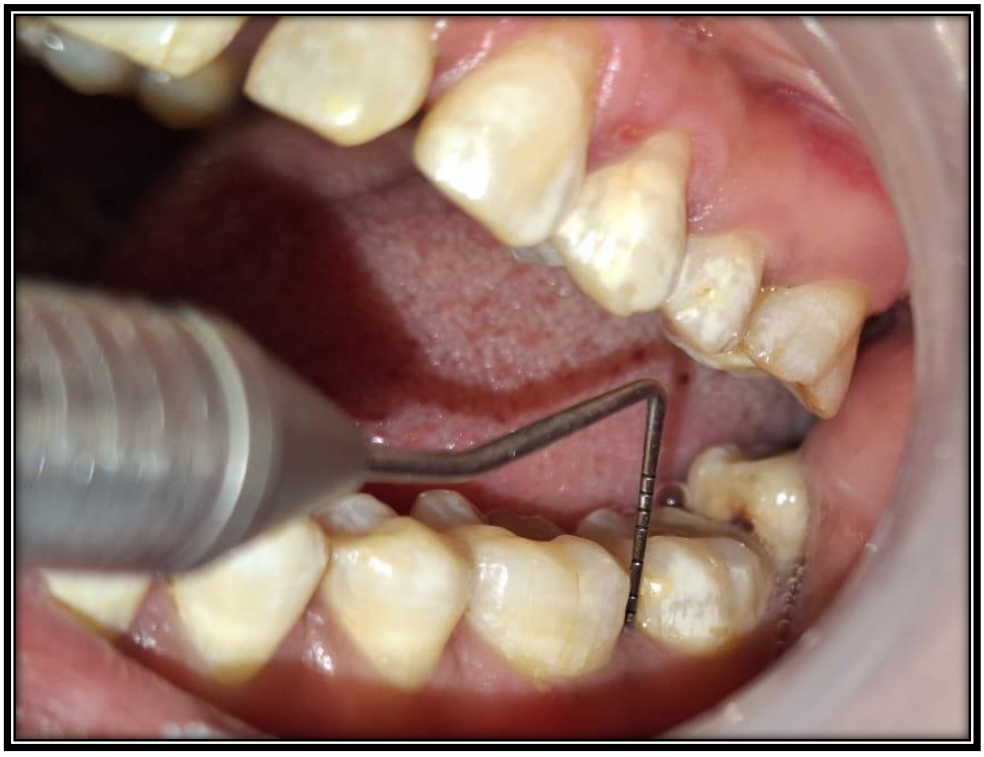


Figure 15: 1 Month Post-Operative for Group 3

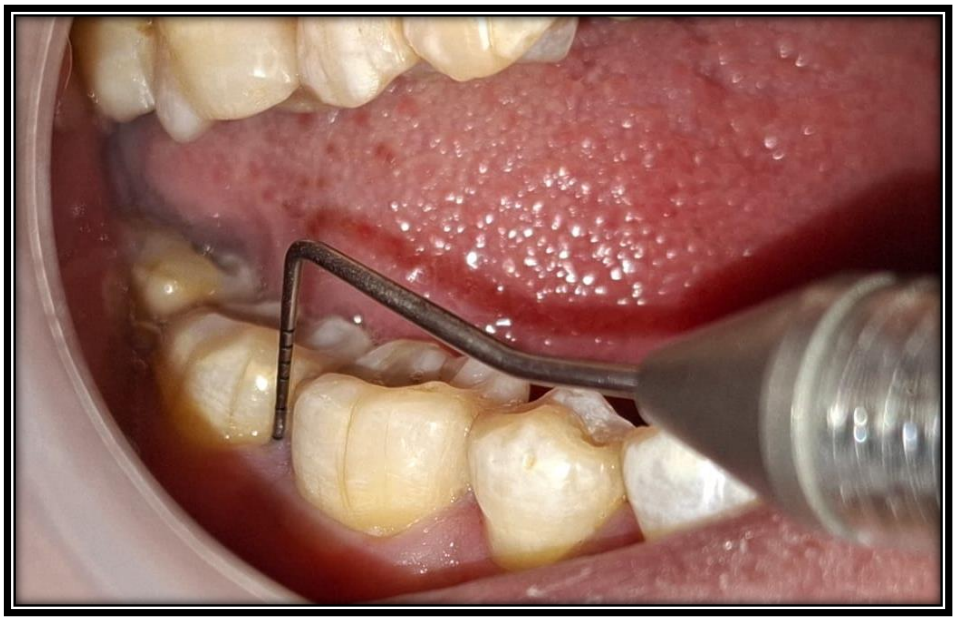
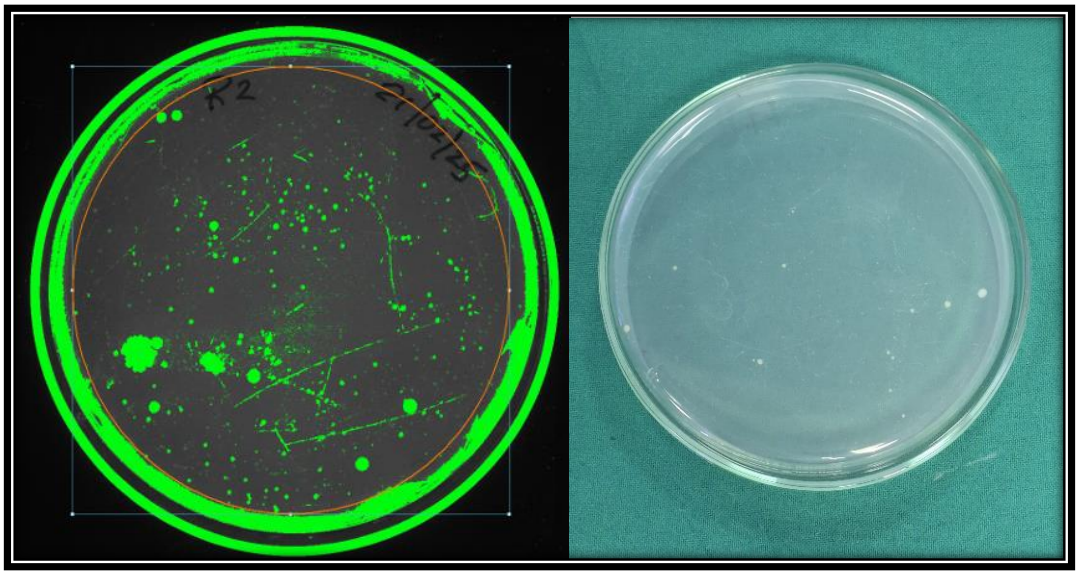


Figure 16: Photograph Showing Agar Plate Inoculated with Subgingival Plaque Sample



RESULTS

Table 1: Demographic Data

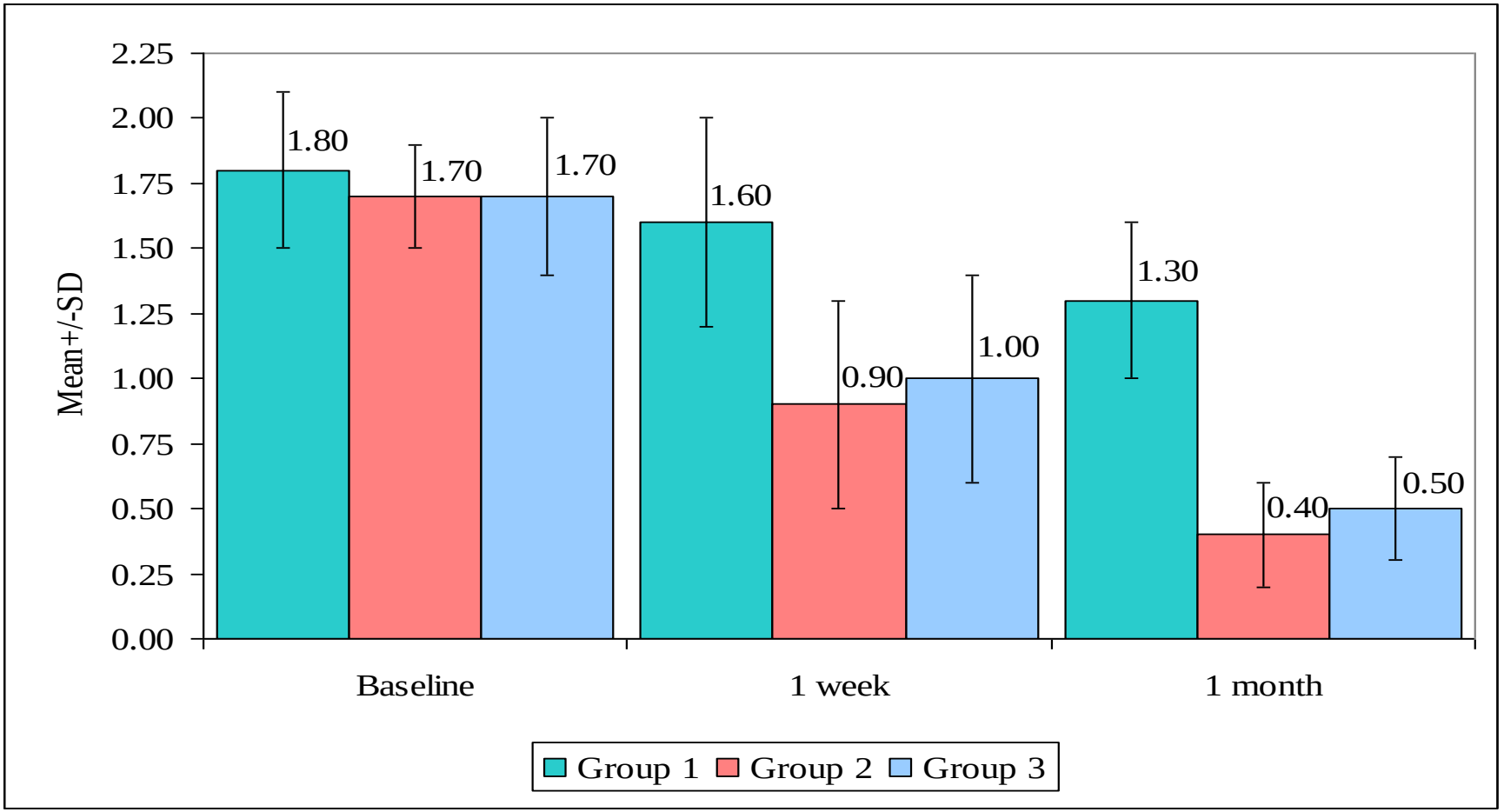
Gender	Male	37.5
	Female	62.5
Age range in yrs		18-50
Age (Mean ± SD) in yrs		35.55 ± 7.79
Total number of teeth included		63
Total number of experimental sites included for clinical parameters		63
Total number of experimental sites included for microbiological parameters		63
Number of subjects with anterior sites		28
Number of subjects with posterior sites		35

Table 2: Comparison of Group 1, Group 2 and Group 3 with gingival index scores at different treatment time points by Kruskal Wallis ANOVA

Treatment time points	Group 1			Group 2			Group 3			H-value	P-value	Pairs by Mann-Whitney U test		
	Mean	SD	Mean rank	Mean	SD	Mean rank	Mean	SD	Mean rank			Grp 1 vs Grp 2	Grp 1 vs Grp 3	Grp 2 vs Grp 3
Baseline	1.8	0.3	21.8	1.7	0.2	21.2	1.7	0.3	21.6	0.0160	0.9920	p=0.8900	p=0.9599	p=0.9599
1 week	1.6	0.4	30.6	0.9	0.4	12.4	1.0	0.4	14.8	25.7790	0.0001*	p=0.0001*	p=0.0004*	p=0.1908
1 month	1.3	0.3	31.3	0.4	0.2	11.7	0.5	0.2	11.9	36.7230	0.0001*	p=0.0001*	p=0.0001*	p=0.3855
Baseline to 1 week	0.2	0.1	12.0	0.8	0.3	31.0	0.7	0.4	29.8	30.7000	0.0001*	p=0.0001*	p=0.0001*	p=0.5714
Baseline to 1 month	0.5	0.3	11.4	1.3	0.3	31.6	1.3	0.4	31.0	36.6230	0.0001*	p=0.0001*	p=0.0001*	p=0.9398
1 week to 1 month	0.3	0.3	17.3	0.5	0.3	25.7	0.6	0.4	25.6	7.6270	0.0220*	p=0.0260*	p=0.0315	p=0.4812

*$p<0.05$

Graph 1: Comparison of Group 1, Group 2 and Group 3 with gingival index scores at different treatment time points



Observations:

At Day 0, gingival index scores for Groups 1 (1.8±0.3), 2 (1.7±0.2), and 3 (1.7±0.3) were comparable. (p=0.9920) The alterations weren't significant statistically.

Gingival index scores showed a significant difference (p=0.0001) between Group 1 (1.6±0.4) and Group 2 (0.9±0.4) when compared from baseline to a 1-week time interval. Gingival index scores showed a significant difference (p=0.0001) between Group 1 (1.6±0.4) and Group 3 (1.0±0.4) when compared from baseline to a 1-week time interval. However, there was no significant difference (p=0.5714) in the GI scores between Group 2 (0.9±0.4) and Group 3 (1.0±0.4) when comparing the GI scores from Day 0 to a one-week interval.

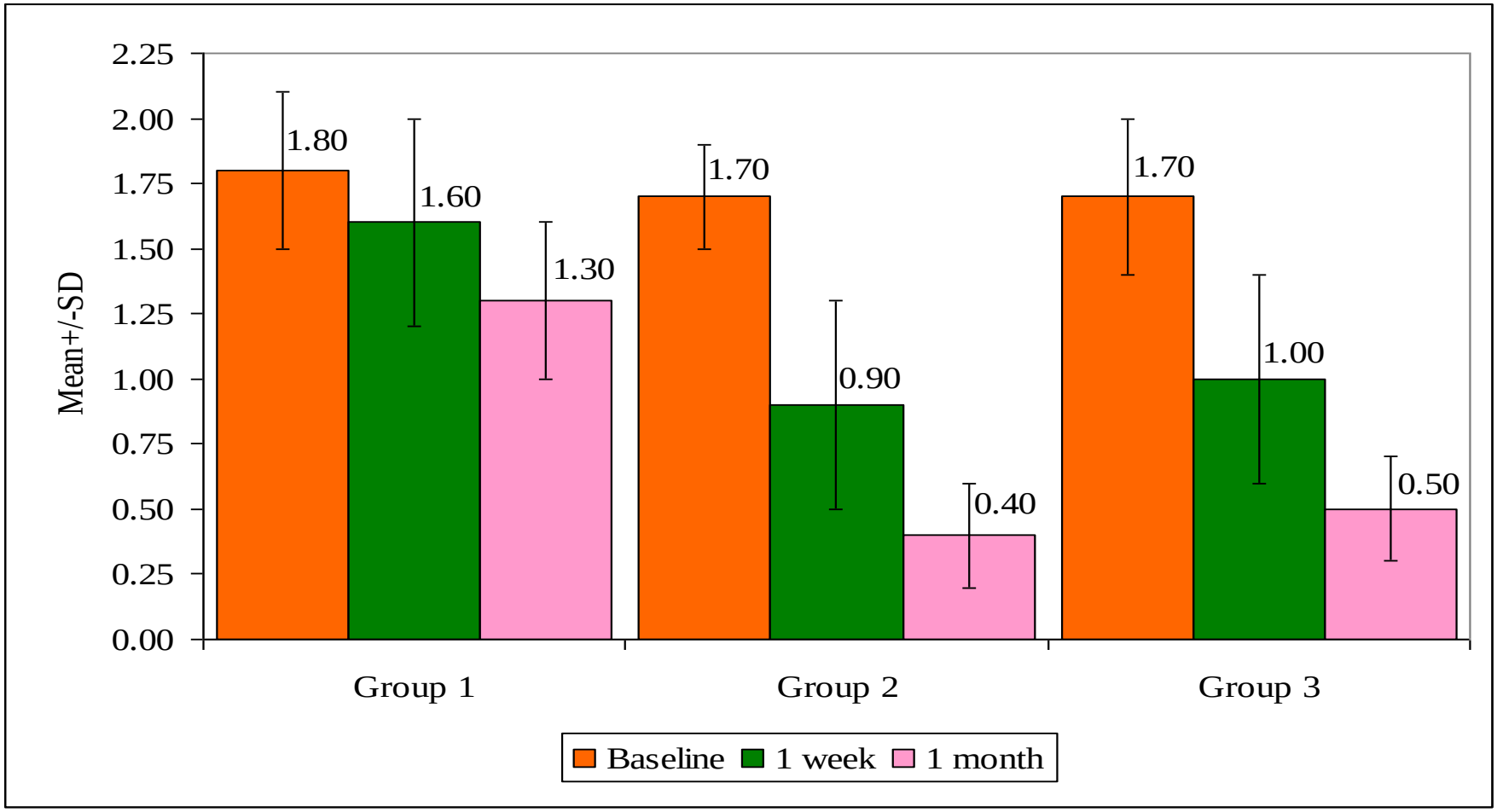
Gingival index scores showed significant difference (p=0.0001) between Group 1 (1.3±0.3) and Group 2 (0.4±0.2) when compared from baseline to a 1-month time interval. Gingival index scores showed a significant difference (p=0.0001) between Group 1 (1.3±0.3) and Group 3 (0.5±0.2) when juxtaposed from Day 0 to a 1-month time interval. However, there was no significant difference (p=0.9398) in the gingival index scores between Group 2 (0.4±0.2) and Group 3 (0.5±0.2) when comparing the gingival index scores from baseline to a one-month interval.

Table 3: Comparison of different treatment time points with gingival index scores in Group 1, Group 2 and Group 3 by Wilcoxon matched pairs test

Group	Changes from	Mean change	% of effect	Z-value	P-value
Group 1	Baseline to 1 week	0.20	11.34	3.7236	0.0002*
	Baseline to 1 month	0.47	26.59	4.0145	0.0001*
	1 week to 1 month	0.27	17.20	3.2958	0.0010*
Group 2	Baseline to 1 week	0.81	47.48	4.0145	0.0001*
	Baseline to 1 month	1.30	76.09	4.0148	0.0001*
	1 week to 1 month	0.49	54.48	3.8230	0.0001*
Group 3	Baseline to 1 week	0.73	41.45	4.0145	0.0001*
	Baseline to 1 month	1.29	73.65	4.0149	0.0001*
	1 week to 1 month	0.56	55.00	3.5162	0.0004*

*$p<0.05$

Graph 2: Comparison of different treatment time points with gingival index scores in Group 1, Group 2 and Group 3



Observations:

Gingival index scores in Group 1 decreased significantly (p=0.0001) from baseline to 1 week (11.34%), from baseline to 1 month (26.59%), and from 1 week to 1 month (17.20%).

Gingival index scores in Group 2 decreased significantly (p=0.0001) from baseline to one week (47.48%), from baseline to one month (76.09%), and from one week to one month (54.48%).

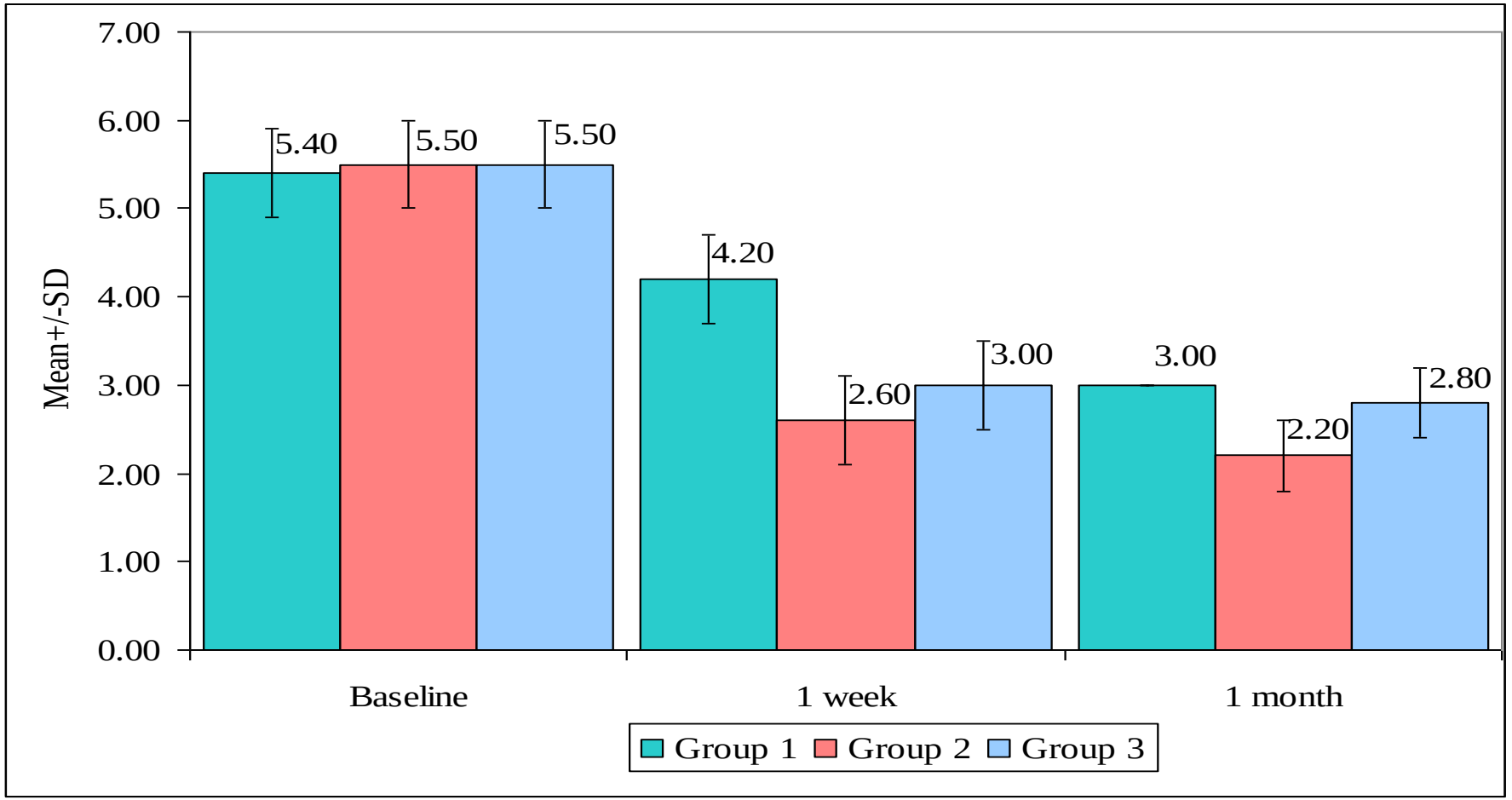
Gingival index values in Group 3 decreased significantly (p=0.0001) from baseline to 1 week (41.45%), from baseline to 1 month (73.65%), and from 1 week to 1 month (55.00%).

Table 4: Comparison of Group 1, Group 2 and Group 3 with Pocket Probing Depth (PPD) scores at different treatment time points by Kruskal Wallis ANOVA

Treatment time points	Group 1			Group 2			Group 3			H-value	P-value	Pairs by Mann-Whitney U test		
	Mean	SD	Mean rank	Mean	SD	Mean rank	Mean	SD	Mean rank			Grp 1 vs Grp 2	Grp 1 vs Grp 3	Grp 2 vs Grp 3
Baseline	5.4	0.5	21.8	5.5	0.5	21.2	5.5	0.5	21.6	0.5060	p=0.7760	p=0.6061	p=0.6061	p=0.9900
1 week	4.2	0.5	30.6	2.6	0.5	12.4	3.0	0.5	14.8	42.5680	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0391*
1 month	3.0	0.0	31.3	2.2	0.4	11.7	2.8	0.4	11.9	28.2590	p=0.0001*	p=0.0001*	p=0.1908	p=0.0038*
Baseline to 1 week	1.2	0.4	12.0	2.9	0.4	31.0	2.4	0.5	29.8	44.5400	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0180*
Baseline to 1 month	2.4	0.5	11.4	3.2	0.6	31.6	2.7	0.5	31.0	18.6810	p=0.0001*	p=0.0003*	p=0.0663	p=0.0180*
1 week to 1 month	1.2	0.5	17.3	0.4	0.5	25.7	0.3	0.5	25.6	25.3600	p=0.0001*	p=0.0003*	p=0.0001*	p=0.6061

*p<0.05

Graph 3: Comparison of Group 1, Group 2 and Group 3 with Pocket Probing Depth (PPD) scores at different treatment time points



Observations:

At Day 0, Group 1 (5.4±0.5), Group 2 (5.5±0.5), and Group 3 (5.5±0.5) all had comparable pocket probing depths. (p=0.7760) The differences were not significant statistically.

There was a significant difference (p=0.0001) in the pocket probing depths between Group 1 (4.2±0.5) and Group 2 (2.6±0.5) when comparing the baseline and 1-week time interval. Group 1 (4.2±0.5) and Group 3 (3.0±0.5) showed significantly different pocket probing depths (p=0.0001) when compared from Day 0 to a 1-week time interval. The pocket probing depths of Groups 2 (2.6±0.5) and 3 (4.0±0.5) differed significantly (p=0.0180) when compared from baseline to a one-week time interval.

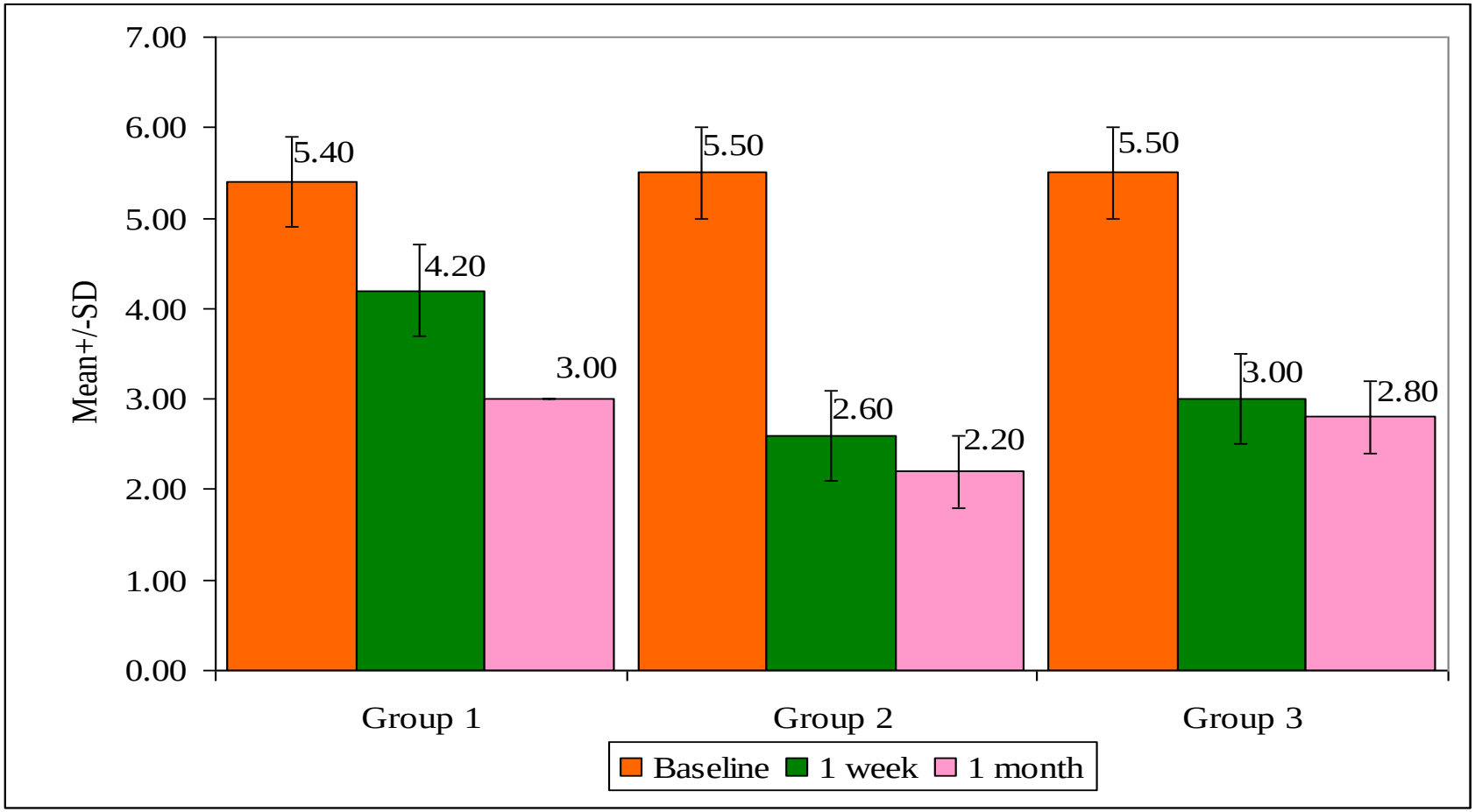
When Group 1 (3.0±0.0) and Group 2 (2.2±0.4) were compared from baseline to a one-month time interval, the pocket probing depths showed a significant difference (p=0.0003). The pocket probing depths of Group 1 (3.0±0.0) and Group 3 (2.8±0.4) did not differ significantly (p=0.0663) when compared from baseline to a one-month time interval. The pocket probing depths of Groups 2 (2.2±0.4) and 3 (2.8±0.4) differed significantly (p=0.0180) when compared from baseline to a one-month time interval.

Table 5: Comparison of different treatment time points with Pocket Probing Depth (PPD) scores in Group 1, Group 2 and Group 3 by Wilcoxon matched pairs test

Group	Changes from	Mean change	% of effect	Z-value	P-value
Group 1	Baseline to 1 week	1.19	22.12	4.0145	0.0001*
	Baseline to 1 month	2.38	44.25	4.0146	0.0001*
	1 week to 1 month	1.19	28.41	3.9199	0.0001*
Group 2	Baseline to 1 week	2.86	52.17	4.0145	0.0001*
	Baseline to 1 month	3.24	59.13	4.0147	0.0001*
	1 week to 1 month	0.38	14.55	2.5205	0.0117*
Group 3	Baseline to 1 week	2.43	44.35	4.0145	0.0001*
	Baseline to 1 month	2.71	49.57	4.0147	0.0001*
	1 week to 1 month	0.29	9.38	2.2014	0.0277*

*$p<0.05$

Graph 4: Comparison of different treatment time points with Pocket Probing Depth (PPD)scores in Group 1, Group 2 and Group 3



Observations:

Pocket probing depths in Group 1 decreased significantly (p=0.0001) from baseline to 1 week (22.12%), from baseline to 1 month (44.25%), and from 1 week to 1 month (28.41%).

Pocket probing depths in Group 2 decreased significantly (p=0.0001) from baseline to 1 week (52.17%), from baseline to 1 month (59.13%), and from 1 week to 1 month (14.55%).

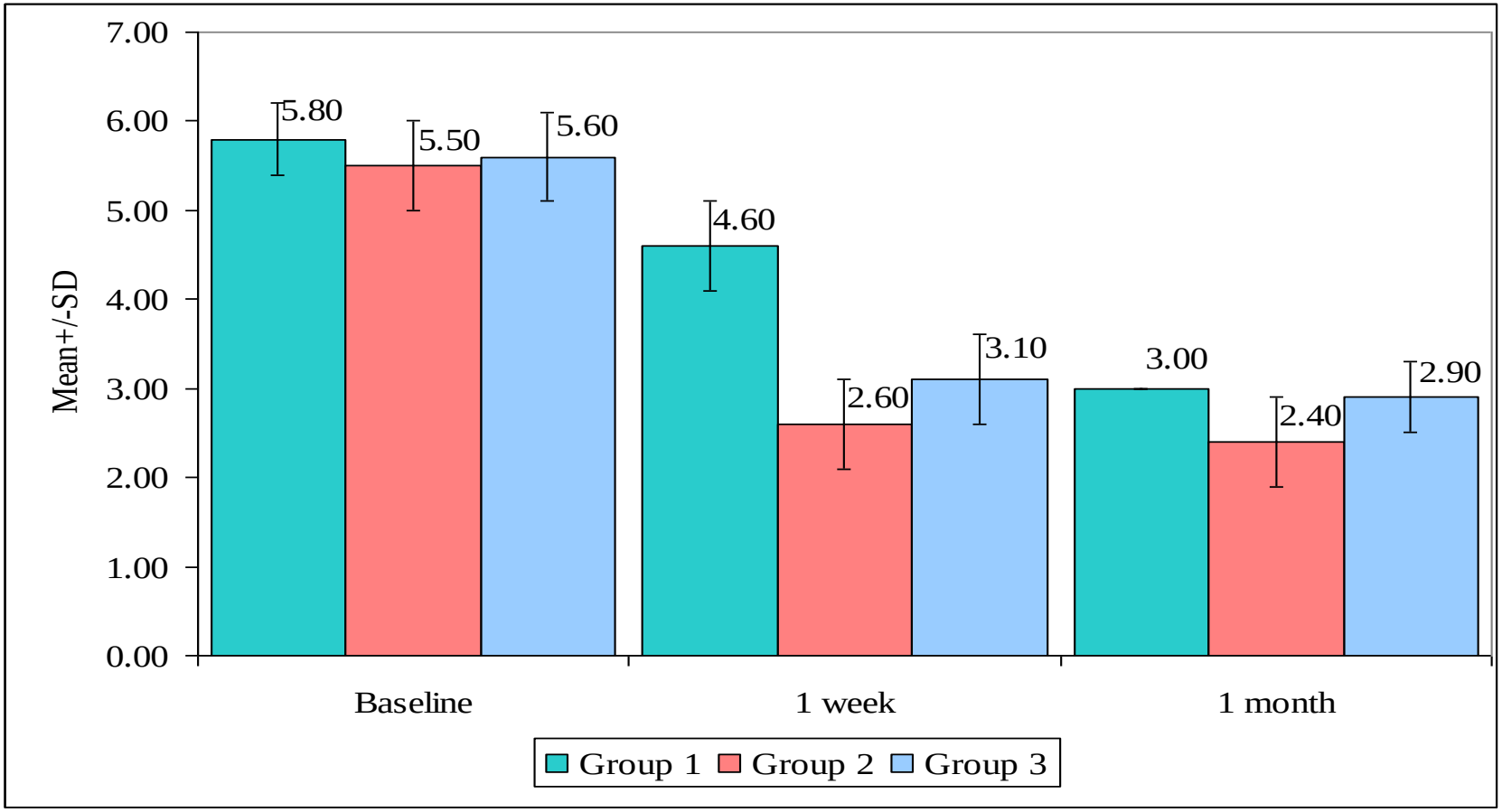
Pocket probing depths in Group 3 decreased significantly (p=0.0001) from baseline to 1 week (44.35%), from baseline to 1 month (49.57%), and from 1 week to 1 month (9.38%).

Table 6: Comparison of Group 1, Group 2 and Group 3 with Clinical Attachment Loss (CAL) scores at different treatment time points by Kruskal Wallis ANOVA

Treatment time points	Group 1			Group 2			Group 3			H-value	P-value	Pairs by Mann-Whitney U test		
	Mean	SD	Mean rank	Mean	SD	Mean rank	Mean	SD	Mean rank			Grp 1 vs Grp 2	Grp 1 vs Grp 3	Grp 2 vs Grp 3
Baseline	5.8	0.4	25.0	5.5	0.5	18.0	5.6	0.5	19.0	5.1670	p=0.0760	p=0.0663	p=0.1908	p=0.6061
1 week	4.6	0.5	32.0	2.6	0.5	11.0	3.1	0.5	11.9	46.8000	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0128*
1 month	3.0	0.0	28.0	2.4	0.5	15.0	2.9	0.4	20.0	22.9200	p=0.0001*	p=0.0006*	p=0.4355	p=0.0086*
Baseline to 1 week	1.2	0.4	11.4	2.9	0.4	31.6	2.4	0.5	30.6	43.2710	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0180*
Baseline to 1 month	2.8	0.4	18.9	3.1	0.5	24.1	2.7	0.6	20.4	5.8220	p=0.0540	p=0.1704	p=0.5714	p=0.0762
1 week to 1 month	1.6	0.5	30.7	0.2	0.5	12.3	0.3	0.5	12.3	37.0950	p=0.0001*	p=0.0001*	p=0.0001*	p=0.8602

*p<0.05

Graph 5: Comparison of Group 1, Group 2 and Group 3 with Clinical Attachment Loss (CAL) scores at different treatment time points



Observations:

At the beginning, Group 1 (5.8±0.4), Group 2 (5.5±0.5), and Group 3 (5.6±0.5) all had comparable levels of clinical attachment loss (p=0.0760). The differences were not statistically significant.

When comparing Group 1 (4.6±0.5) and Group 2 (2.6±0.5) from baseline to a one-week interval, there was a significant difference (p=0.0001) in the CAL. Group 1 (4.6±0.5) and Group 3 (3.1±0.5) showed a significant difference (p=0.0001) in CAL when compared from baseline to a 1-week interval. Group 2 (2.6±0.5) and Group 3 (3.1±0.5) showed a significant difference (p=0.0180) in CAL when compared from baseline to a 1-week time period.

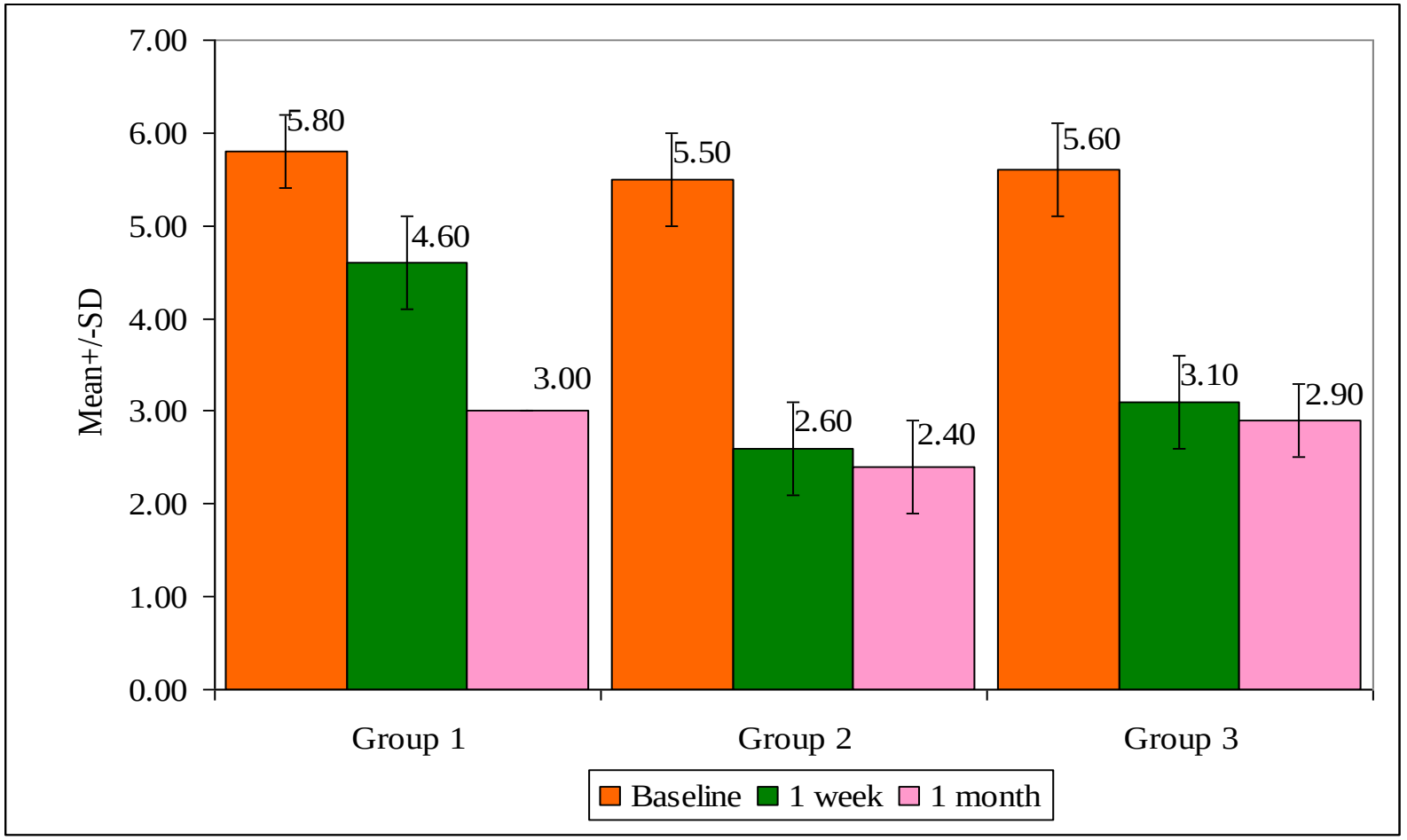
Group 1 (3.0±0.0) and Group 2 (2.2±0.4) did not vary significantly (p=0.1704) in terms of clinical attachment loss when compared from baseline to a one-month interval. When comparing Group 1 (3.0±0.0) and Group 3 (2.9±0.4) from Day 0 to a one-month interval, there was no discernible difference in the CAL (p=0.5714). The clinical attachment loss did not significantly differ (p=0.0762) between Group 2 (2.2±0.4) and Group 3 (2.9±0.4) when compared from baseline to a one-month interval.

Table 7: Comparison of different treatment time points with Clinical Attachment Loss (CAL) scores in Group 1, Group 2 and Group 3 by Wilcoxon matched pairs test

Group	Changes from	Mean change	% of effect	Z-value	P-value
Group 1	Baseline to 1 week	1.24	21.31	4.0145	0.0001*
	Baseline to 1 month	2.81	48.36	4.0148	0.0001*
	1 week to 1 month	1.57	34.37	4.0140	0.0001*
Group 2	Baseline to 1 week	2.86	52.17	4.0144	0.0001*
	Baseline to 1 month	3.10	56.52	4.0145	0.0001*
	1 week to 1 month	0.24	9.09	1.6903	0.0910*
Group 3	Baseline to 1 week	2.43	43.59	4.0143	0.0001*
	Baseline to 1 month	2.71	48.72	4.0149	0.0001*
	1 week to 1 month	0.29	9.09	2.2014	0.0277*

*$p<0.05$

Graph 6: Comparison of different treatment time points with Clinical Attachment Loss (CAL) scores in Group 1, Group 2 and Group 3



Observations:

The clinical attachment loss was significantly lower in Group 1 from baseline to 1 week (21.31%), from baseline to 1 month (48.36%), and from 1 week to 1 month (34.37%) (p=0.0001).

The clinical attachment loss in Group 2 was significantly lower (p=0.0001) from baseline to 1 week (52.17%), from baseline to 1 month (56.52%), and from 1 week to 1 month (9.09%).

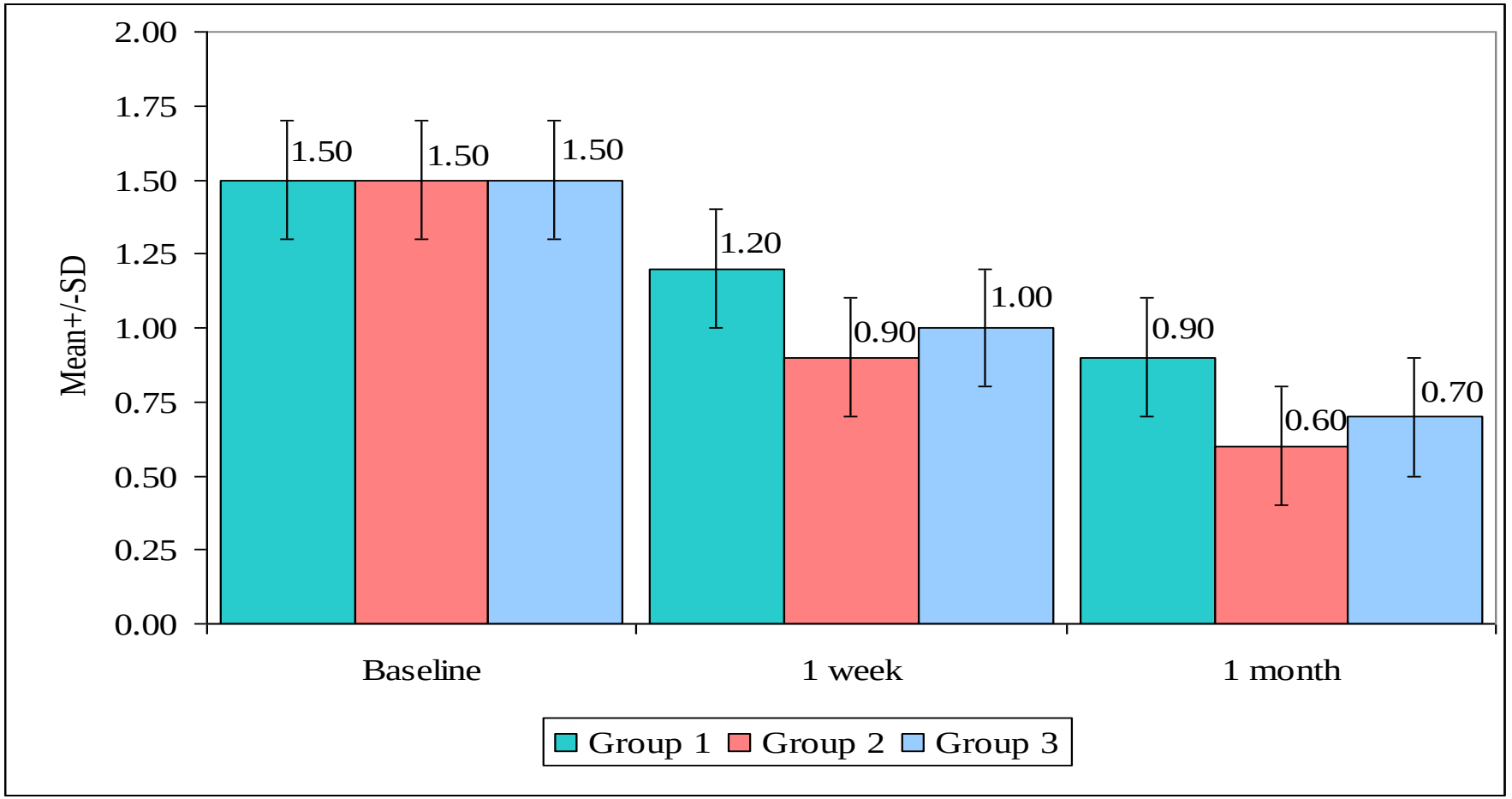
The clinical attachment loss in Group 3 was significantly lower (p=0.0001) from baseline to 1 week (43.59%), from baseline to 1 month (48.72%), and from 1 week to 1 month (9.09%).

Table 8: Comparison of Group 1, Group 2 and Group 3 with log (CFU/ml) counts at different treatment time points by Kruskal Wallis ANOVA

Treatment time points	Group 1			Group 2			Group 3			H-value	P-value	Pairs by Mann-Whitney U test		
	Mean	SD	Mean rank	Mean	SD	Mean rank	Mean	SD	Mean rank			Grp 1 vs Grp 2	Grp 1 vs Grp 3	Grp 2 vs Grp 3
Baseline	1.5	0.2	22.2	1.5	0.2	20.8	1.5	0.2	20.8	0.1990	p=0.9050	p=0.7153	p=0.7153	p=0.9099
1 week	1.2	0.2	29.0	0.9	0.2	14.0	1.0	0.2	15.8	18.3140	p=0.0001*	p=0.0001*	p=0.0026*	p=0.1312
1 month	0.9	0.2	30.2	0.6	0.2	12.8	0.7	0.2	15.9	25.0480	p=0.0001*	p=0.0001*	p=0.0032*	p=0.0052*
Baseline to 1 week	0.3	0.0	11.0	0.5	0.0	32.0	0.4	0.0	32.0	52.3900	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*
Baseline to 1 month	0.6	0.1	11.0	0.9	0.1	32.0	0.7	0.1	30.2	46.1250	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*
1 week to 1 month	0.3	0.1	14.2	0.4	0.1	28.8	0.3	0.1	19.3	21.4910	p=0.0001*	p=0.0001*	p=0.2524	p=0.0001*

*p<0.05

Graph 7: Comparison of Group 1, Group 2 and Group 3 with log (CFU/ml) counts at different treatment time points



Observations:

Group 1 had 1.5±0.2 colony forming units per milliliter at baseline, while Group 2 had 1.5±0.2 and Group 3 had 5.6±0.5. (p=0.9050). The alterations weren't significant statistically.

Number of colony forming units per milliliter varied significantly (p=0.0001) between Group 1 (1.2±0.2) and Group 2 (0.9±0.2) from baseline to a one-week time interval. The number of colony forming units per milliliter varied significantly (p=0.0001) between Group 1 (1.2±0.2) and Group 3 (1.0±0.2) when compared from baseline to a one-week time interval. The number of colony forming units per milliliter varied significantly (p=0.0001) between Groups 2 (0.9±0.2) and 3 (1.0±0.2) when compared from baseline to a one-week time interval.

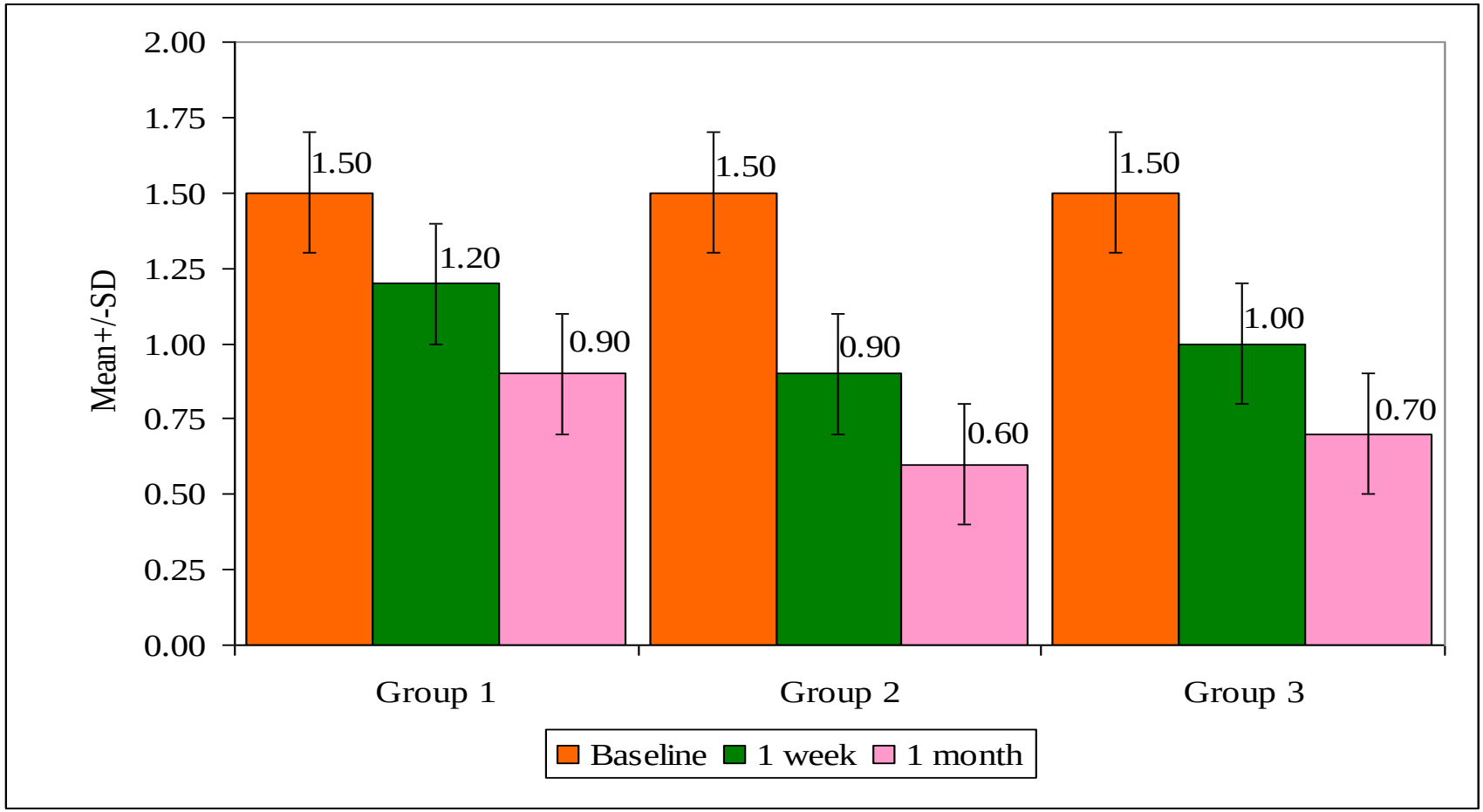
The number of colony forming units per milliliter varied significantly (p=0.0001) between Group 1 (0.9±0.2) and Group 2 (0.6±0.2) when compared from baseline to a one-month time interval. The number of colony forming units per milliliter varied significantly (p=0.0001) between Group 1 (0.9±0.2) and Group 3 (0.7±0.2) from baseline to a one-month time interval. The number of colony forming units per milliliter varied significantly (p=0.0001) between Groups 2 (0.6±0.2) and 3 (0.7±0.2) from baseline to a one-month time interval.

Table 9: Comparison of different treatment time points with log (CFU/ml) counts in Group 1, Group 2 and Group 3 by Wilcoxon matched pairs test

Group	Changes from	Mean change	% of effect	Z-value	P-value
Group 1	Baseline to 1 week	0.25	17.22	4.0145	0.0001*
	Baseline to 1 month	0.56	38.45	4.0148	0.0001*
	1 week to 1 month	0.31	25.64	4.0144	0.0001*
Group 2	Baseline to 1 week	0.51	34.91	4.0145	0.0001*
	Baseline to 1 month	0.90	61.55	4.0146	0.0001*
	1 week to 1 month	0.39	40.93	4.0148	0.0001*
Group 3	Baseline to 1 week	0.43	29.62	4.0142	0.0001*
	Baseline to 1 month	0.73	49.92	4.0147	0.0001*
	1 week to 1 month	0.30	28.84	4.0143	0.0001*

*$p<0.05$

Graph 8: Comparison of different treatment time points with log (CFU/ml) counts in Group 1, Group 2 and Group 3



Observations:

Number of colony forming units per milliliter decreased significantly (p=0.0001) in Group 1 from baseline to one week (17.22%), from baseline to one month (38.45%), and from one week to one month (25.64%).

The number of colony forming units per milliliter decreased significantly (p=0.0001) in Group 2 from baseline to 1 week (34.91%), from baseline to 1 month (61.55%), and from 1 week to 1 month (40.93%).

The number of colony forming units per milliliter decreased significantly (p=0.0001) in Group 3 from baseline to one week (29.62%), from baseline to one month (49.92%), and from one week to one month (28.84%).

DISCUSSION

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more so that we may fear less."

- Marie Curie.

Periodontitis, a chronic inflammatory disorder of the periodontium attributed to pathogenic interactions between virulent bacteria and the human response, is the 6th prevalent condition in the world. It has been implicated to the disease progression of several additional systemic inflammatory disorders, notably rheumatoid arthritis, type 2 diabetes, and persistent renal failure. The ultimate result of the condition is loss of periodontal attachment.[39]

Initial periodontal treatment must include SRP. However, the conventional NSPT may be challenging to implement in areas with complex anatomy characteristics such as concavities, furca, grooves, and extensive pockets, which reduces or eliminates the periodontal pathogens. With differing degrees of efficacy, a variety of adjuvant medications, such as systemic and local antibiotics, have been employed in concert with mechanical debridement to enhance NSPT results.[40]

Research has been done on the use of several adjuvant modalities to treat periodontitis. The therapeutic efficacy of antibiotics, whether given locally or systemically, has been shown to range from 0.40 mm to more than 0.80 mm in PPD decrease. The dangers associated with antibiotics, such as the potential for anaphylaxis, antimicrobial resistance, and the need for high dosages when administered systemically, severely restrict their use.[41]

One potential alternative to periodontal therapy is aPDT, which is predicated on the notion that a photosensitizer can produce oxygen in singlet form along with ROS that are exceedingly harmful to microbes and their output when exposed to the right wavelength of light. PDT's bactericidal and detoxifying properties have made it a non-invasive therapeutic approach for the treatment of a variety of bacterial, fungal, and viral ailments. The main advantages of aPDT are its sizeable array of pursuit with preferrable restraining of microorganisms, which reduces adverse outcomes; its multi-target approach and non-target selectivity in microorganisms, which prevents the development of antimicrobial resistance; and its straightforward and controllable singlet oxygen production method, which makes it a low-risk, highly ergonometric treatment.[5]

Chemical compounds that easily undergo photoexcitation and subsequently transfer their energy to other molecules are known as photosensitizers. Numerous chemical substances that exhibit exceptional photosensitizing qualities have been investigated and are now being applied in a number of medicinal specialties. Few, however, are suitable for use as antibacterial treatment. Toluidine blue, MB, porphyrin, and their derivatives are the most often used photosensitizers in periodontal aPDT; nonetheless, the transmission of energy and light stimulation of these substances require visible spectrum light. Within biological tissues, wavelengths in the specified spectrum, namely 390–700, have a restricted penetrating potential. One of the most recent photosensitizers is ICG, a tri-carbo cyanine dye that reaches its maximum attenuation at about 800 nm in the infra-red band.[42]

Despite both dyes being used as photosensitizers in dentistry due to conflicting findings from studies examining their effects on moderate periodontitis, the present research sought to juxtapose antimicrobial outcomes of MB and ICG dye as PS for aPDT as an additive to SRP in patients with moderate periodontal disease.

A single-blinded RCT was conducted on sixty-three cases with moderate chronic periodontal disease who visited the outpatient Department of Periodontics at KLE V.K. Institute of Dental Sciences, Belagavi. To eliminate influence of inter-examiner variability all the measurements were taken by a "blinded," calibrated operator. Under local anaesthetic, patients in Group 1 (n = 21) received SRP. After SRP, the periodontal pockets in Group 2 (n = 21) were exposed to aPDT using a 0.5% Indocyanine green solution. After SRP, the periodontal pockets in Group 3 (n = 21) underwent aPDT with 1% Methylene blue solution. The patients' GI scores, PPD, CAL, and nCFU/ml were assessed at Day 0, 7 days and 1 month duration.

Numerous lasers, photosensitizing chemical combinations have been used in antimicrobial photodynamic therapy. In 2011, Aykol et al.[43] used a GaAlAs 808 nm diode laser with a power of 0.25 W to perform a randomized clinical trial. Polansky et al.[44] carried out a second study in 2009 with a 680 nm diode laser with a power of 75 W.

In contrast to a 660 nm laser, it was found that, one with a wavelength of 808 nm yielded superior results and was more effective against a variety of periodontopathogens.[45] Since diode laser is found to be effective for photodynamic therapy, the current study used a multifiber optic tip to operate an IndiLase Soft tissue laser (660 nm, 810 nm) in continuous mode for 60 seconds at a peak output of 0.1 W and 6 Joules per site.

aPDT has usually been evaluated in conjunction with traditional photosensitizing chemicals like methylene blue and toluidine blue. These traditional agents seem to have little therapeutic utility and work by photochemical methods. With the potential for improved periodontal disease treatment efficacy, modern photosensitizing agents have recently been devised. Indocyanine green is one such photosensitizing agent that is frequently studied. Unlike conventional agents, which work by photochemical methods, this anionic photosensitizer exhibits its effects predominantly through photothermal activity and has a higher peak absorption than traditional agents.[46]

Furthermore, it has been demonstrated in vitro that ICG is particularly effective in eradicating microorganisms that are strongly linked to periodontitis, including *P. gingivalis* and *A. actinomycetemcomitans*. Additionally, ICG-PDT effectively eradicates strains of common bacterial species that are resistant to antibiotics. When taken as a whole, this data suggests that ICG-PDT may offer therapeutic advantages in situations that are beyond the purview of traditional antibiotics.[47] In order to improve clinical parameters and reduce periodontopathogens microbiologically, the current study compared a 0.5% Indocyanine Green solution activated at 810 nm with a 1% Methylene Blue solution activated at 660 nm.

The gingival index reflects the clinical condition of the tissues (Loe H and Silness J, 1963), which is based on the hallmarks of inflammation: redness, edema, and bleeding.[48] When intra-group comparisons were performed in this study, all three groups showed a substantial refinement from Day 0 to 7- and 1-month ($p=0.0001$). When supragingival and subgingival bacterial deposits were removed from the control group, dental plaque was disrupted through scaling and root planing.

This was accompanied by histological changes, such as a reduction in the size of the inflammatory infiltrate. This reduces inflammation, which limits the disease's progression and lessens gingival bleeding.[48]

Intergroup comparisons revealed that Group 1 and Group 2 (p=0.0001) and Group 1 and Group 3 (p=0.0001) had significantly improved from Day 0 to 7 and 1 month, in contrast to the alterations amongst Group 2 and Group 3 (p=0.5714) wasn't statistically drastic. By lowering gingival inflammation, PDT's well-known anti-inflammatory properties might have helped the test groups' gingival health. PDT may lessen gingival tissue inflammation by increasing anti-inflammatory cytokine levels and decreasing the generation of pro-inflammatory mediators like TNF-α and IL-1β.[49] A single photodynamic therapy session, when added to SRP, greatly enhanced clinical parameters such gingival index, per studies by Braun et al. (2009)[50], Theodoro et al. (2012)[51], and Ravi et al. (2016)[52].

The measurement of pocket probing depth is a crucial part of a periodontal examination. It indicates how much periodontal support is still there around the tooth and evaluates the extent of periodontal tissue deterioration. It is advantageous to decrease the depth of pocket probing because this creates an environment that is less conducive to the growth of anaerobic periodontopathic microorganisms. Additionally, lower PPD values make it easier to remove plaque during self-performed dental hygiene and to access later debridement and polishing during the perpetuation phase of supportive periodontal therapy.[53]

Intragroup comparisons in the current study showed that all three groups significantly improved from Day 0 to 7 and 1 month (p=0.0001). In terms of the inter-group comparison, Group 1 and Group 2 demonstrated a statistically significant alteration from baseline to one week and one month, while Group 1 and Group 3 demonstrated an insignificant difference from baseline to one month. When Group 2 was compared to Group 3, the differences from Day 0 to 7 and 1 month were statistically significant, indicating that PDT performed with 0.5% Indocyanine green solution produced better results than with 1% Methylene blue solution (Table 4). These findings align with a study by Monzavi et al. that discovered that ICG mostly has a photothermal therapeutic impact (80% photothermal and 20% photochemical). Additionally, the peak absorption of ICG is close to the 800 nm range of dental diode lasers that are now on the market; in comparison, the highest absorptions of MB and toluidine blue O are 660 nm and 635 nm, respectively.[28]

Accessing deep pockets is made easier by the 810 nm diode laser's greater penetration than other wavelengths and the ease with which the fiber-optic applicator can be inserted. Additionally, ICG's photothermal and photochemical actions make it a valuable photosensitizer for treating non-reachable areas of the mouth or for annihilating infections in periodontal pockets that are fairly deep.[47]

For additional periodontal treatment, ICG is a good substitute for widely used photosensitizers such as MB and toluidine blue O. Similar experiments were put forward by Campos et al.[15] as well as Lui et al.[14] using MB as a photosensitizer and Sethi et al.[31] and Raut et al.[32] using ICG as a photosensitizer.

According to the findings of both investigations, the test group's PPD significantly decreased in comparison to the control group. Ruhling et al.[54], de Oliveira RR et al.[55], and Al Zahrani MS et al.[56], however, conducted research that refuted the changes in PPD decrease and came to the conclusion that PDT and SRP had no further advantages over SRP. They claimed that one of the possible causes of PDT's lack of effectiveness could be the brief laser exposure. However, it is important to remember that it is challenging to compare research due to differences in diagnosis of periodontal disease, research outline, photosensitizer variety and concentration, laser type, configuration, and application technique. After six months of SPT, Lulic et al. (2009)[57] examined the effects of recurrent doses of PDT with SRP in remainder pockets of patients and discovered that five sessions of PDT combined with SRP significantly improved the test group's PD, CAL, and BOP.

Clinical attachment loss is a crucial metric for evaluating treatment outcomes, asserts Killoy et al. (2002)[58]. The percentage of sites that have improved by more than two milli meters in CAL and the number of areas that still need treatment are crucial in this research. According to the current study's intergroup comparison, Group 1 and Group 2 and Group 1 and Group 3 had drastically different CALs from baseline to one week, but the changes were negligible from Day 0 to 1 month (Table 6). When comparing the CAL for each of the three groups intragroup Ly, there was a statistically significant improvement from Day 0 to 7 and 1 month (p=0.0001).

These results are in congruence to research put across by Monzavi et al.[28], who found no discernible improvements from baseline to one- and three-month time intervals.

In contrast, research by Sethi et al.[31] and Andersen et al.[5] revealed that the SRP plus PDT group significantly increased their CAL compared to the SRP and PDT alone groups. Regarding the attachment gain following PDT application, there is still some debate, nevertheless. Numerous studies have come to the conclusion that PDT has no bearing on attachment gain. Since there was clinically insignificant gingival recession, the increase in attachment must be the product of a decrease in PD.

We are unable to comment on the sort of attachment that happened, despite the fact that PD decreased and CAL increased. Nevertheless, aside from other contributing elements like the elimination of local causes and the ensuing decrease in inflammation, it is most likely caused by the establishment of long epithelium.

The analysis of microbial colony forming units per ml was done using spread plate method since it is the most commonly used method to detect and quantify major components of the subgingival plaque. This method's ability to identify several bacterial species at once and the potential to acquire both absolute and relative counts of cultivated species are its primary advantages (Sanz et al., 2004; Socransky et al., 1998)[59]. When comparing all groups intragroup Ly, there was a drastic reduction from Day 0 to 7 and one month (p=0.0001).

Just SRP leads in a drastic change in the subgingival microbiota comparison. It makes it possible to remove endotoxins and calculus from the root surface, which disrupts the subgingival biofilm and creates an environ instrumental in growth of good bacteria. Group 1 and Group 2, Group 1 and Group 3, and Group 2 and Group 3 all had significantly lower CFU/ml when compared across groups (Table 8).

Raut et al.[32] claimed in their study that colony forming units significantly decreased as a result of both SRP and PDT. Nevertheless, there was a greater mean drop in CFU/ml in the test group. These results are congruent to previous studies carried out by Srikanth et al.[27], which stated the anaerobic pathogen levels to be much lower in the PDT and laser groups.

This suggests that there are now less anaerobic floras overall. Even though our estimate was quantitative, it is nevertheless noteworthy because a reduction in the total anaerobic bacterial load is a major factor in periodontal health. The deadly effects of photodynamic inactivation on bacterial cells have been attributed to two basic mechanisms: (i) damage to DNA, and (ii) destruction that occurs in the cytoplasmic membrane, which either inactivates membrane transport systems and enzymes or allows cellular contents to leak out.

According to Hill et al.'s[36] research, the shift to a more anaerobic environment is made possible by periodontal pockets with deeper probing depths. Under anaerobic conditions, the calcium concentration significantly increased, according to elemental composition analysis. Bacterial cells absorb less of the photosensitizer MB intracellularly when bivalent cations are present. Conversely, gram -ve and +ve bacteria are both prompted to absorb more ICG by bivalent cations.

The use of MB-based aPDT may therefore be restricted due to the high mineral content in deep periodontal pockets; on the other hand, the use of an anionic photosensitizer, such as ICG, may be recommended in its place. ICG-based photosensitizers naturally contribute to a patient's increased comfort because they cause less coloring of the oral structures than phenothiazine dyes (such MB).

ICG has several advantages over MB, including minimal toxicity, no side effects, ease of handling for both the patient and the practitioner, an absorption peak near the emission maximum of the dental diode lasers that are currently available in the market (about 800 nm), and quick elimination.

LIMITATIONS AND FURTHER SCOPE

Despite the fact that this randomized controlled clinical trial offers important information about the relative antimicrobial effectiveness of MB and ICG as photosensitizers in aPDT as an additive to SRP, the examiner wasn't blinded for the test and control sites because this study was single blinded. The results might have been biased as a result of this.

The study's sample size is modest; a bigger sample size would be necessary to examine the effects of PDT and the variations in the application of different photosensitizers in a more thorough way. The follow-up time in our study was not very long. Nonetheless, Boehm and Ciancio's[8] findings showed that ICG might significantly improve bacterial killing in a brief amount of time. Therefore, to evaluate the relative effects of ICG and MB as photosensitizers, further RCT's with a greater sample size and an extended study duration is the need of the hour.

There is miniscule literature owing to the efficacy of aPDT against particular bacterial strains because the current study concentrated on measuring the total number of colony forming units. Additionally, there are currently no set standards for the concentration of photosensitizer, duration of incubation, light source, rinse procedure, irradiation method, scheduling, and number of sessions for aPDT in periodontal treatments.

We suggest conducting extended follow-up studies that involve additional patients and study sites in order to assess aPDT's potential for treating CGP as well as developing a standard methodology for the best therapeutic benefit.

Furthermore, its effects can be assessed in patients with systemically compromised persons, advanced periodontitis, and peri-implantitis. Furthermore, different investigations may employ one or many PDT applications. Researchers have demonstrated that in order to guarantee that the clinical outcomes are noticeable, it is essential to administer repeated applications, Lulic et al.[57] discovered that when PDT was used five times in conjunction with mechanical periodontal therapy, clinical results in remainder pockets following SPT improved. The current trial demonstrated that 1 session of aPDT in addition to NSPT was effective in enhancing encouraging changes in clinical aspects in contrast to SRP alone. Depending on this, variations in PDT's laser intensity settings, contact duration, number of applications, and other elements may affect how well certain studies control periodontal disease. Future research can strengthen the validity and relevance of the findings in our study by addressing these limitations.

SUMMARY AND CONCLUSION

In order to evaluate and compare the antibacterial properties of 1% methylene blue dye and 0.5% indocyanine green dye as photosensitizers for aPDT as a supplement to SRP in patients with moderate periodontitis, the current study was conducted. This study was carried out at the KLE V.K Institute of Dental Sciences, Belagavi, at the Outpatient Periodontics Department.

63 patients diagnosed as having moderate periodontitis were selected for the randomized, controlled, single-blinded clinical trial. They were then randomly allocated amongst three groups. Group 1 (n = 21) was the control group whereby NSPT was performed using ultrasonic devices under local anesthesia. In Group 2 (n = 21), 0.5% Indocyanine green solution was flushed into the periodontal pockets and in Group 3 (n = 21), 1% Methylene blue solution was flushed into the periodontal pockets followed by which aPDT was performed in Group 2 and 3 at wavelengths 810 nm and 660 nm respectively with an InGaAs semiconductor diode laser at a power of 0.1 W (6 Joules) using a multifiber tip for 60 seconds. At Day 0 to 7- and 1-month intervals, clinical parameters were assessed using PPD, GI and CAL, whereas microbiological parameters were assessed using nCFU/ml.

Considering the study's limitations, the following findings were arrived at:

When juxtaposing Groups 1 and 2, as well as Groups 1 and 3, NSPT and aPDT were efficacious in improving the GI from Day 0 to 7 and 1 month. However, there were no drastic changes between Groups 2 and 3.

As an adjuvant treatment, aPDT also successfully decreased the depths of the probing pockets from baseline to one week when juxtaposing Groups 1 and 2 as well as Groups 1 and 3 and Group 2 and 3. Although, from baseline to one month, there were significant differences amongst Groups 1 and 2 and Group 2 and 3, there weren't any drastic alterations amongst Groups 1 and 3.

Juxtaposing Groups 1 and 2, Groups 1 and 3, and Groups 2 and 3, aPDT, as an adjuvant treatment, was successful in raising clinical attachment levels from Day 0 to 7; however, from Day 0 to 1 month, the differences between the three groups were negligible. From Day 0 to 7 and Day 0 to 1 month time intervals, a notable drop in nCFU/ml amongst the three groups was depicted.

In conclusion, our investigation into the comparative antimicrobial efficacy of ICG and MB as photosensitizers for aPDT yielded promising insights for combating moderate periodontitis as an adjunct to conventional ultrasonic SRP. The synergistic activity observed between improvement of microbiological and clinical parameters with aPDT emphasizes the potential of combining antimicrobial therapies to enhance the treatment outcomes for patients diagnosed with moderate periodontitis.

In contrast to traditional photosensitizers like Methylene blue, which have only moderately benefited as a supplement, our results indicate that the employment of Indocyanine green mediated aPDT proves to be a strong antibacterial agent, facilitating prolonged antimicrobial activity and offers a non-invasive and targeted approach to bacterial eradication, harnessing the oxidative power of light-activated photosensitizers to disrupt bacterial membranes and cellular structures.

Future studies in longitudinal study models, including experimental studies with greater number of samples and extended periods of monitoring, and multiple sessions of aPDT need to be carried out to validate the observations seen in our study. Strengthening our comprehension of the intricate interplay between biomaterials, microbial pathogens, and antimicrobial therapies, we can pave the way for innovative approaches to improve the success rates of NSPT to treat moderate cases of periodontitis, ultimately benefiting patient outcomes and quality of life.

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ANNEXURES

ANNEXURE 1: ETHICAL CLEARANCE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
Accredited 'A' Grade by NAAC — Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 — Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 — E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1639

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of antimicrobial effect of indocyanine green and methylene blue dye as photosensitizers for photodynamic therapy as an adjunct to scaling and root planing in patient with moderate periodontitis - A randomized controlled trial

Submitted by

Dr. __ **REG NO. IK0222002** _____ *P. G. Student / Staff, Guided by* _____ *from Department of* Periodontics _____ *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 9/12/22

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE 2: COLONY FORMING UNIT – RESULT

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; **Web**: www.klepkbsrc.org, **Phone**: 0831- 2444444, Extn. 4122

Report **Date: 29-03-2025**

Title of Research: Comparative Evaluation of Antimicrobial Effect of Indocyanine Green and Methylene Blue Dye as Photosensitizers for Photodynamic Therapy as an Adjunct to Scaling and Root Planing in Patients with Moderate Periodontitis - A Randomized Controlled Trial

Student Name:] **REG NO. IK0222002**

Guide:]

Co-Guide:

Objective Parameters

1. To assess the antimicrobial efficacy of photodynamic therapy with 0.5% Indocyanine green dye as an adjunct to scaling and root planing.
2. To assess the antimicrobial efficacy of photodynamic therapy with 1% Methylene blue dye as an adjunct to scaling and root planing.
3. To compare the antimicrobial efficacy of 0.5% Indocyanine green dye and 1% Methylene blue dye as photosensitizers for photodynamic therapy.

Experimental Methodology

Subgingival plaque samples were collected at baseline, 1 week and 1 month time intervals for both the test as well as control group with a sterile Gracey's curette. The samples were collected in a sterilized microcentrifuge tube containing thioglycolate broth (Transport media) and transported to KAHER's Dr. Prabhakar Kore Basic Research Center, Belagavi for microbial evaluation within 20 minutes from collection.

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DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; **Web**: www.klepkbsrc.org, **Phone**: 0831- 2444444, Extn. 4122

1. Sample Preparation and Serial Dilution

A sterile nutrient agar medium was prepared and poured it into sterile petri dishes and allowed to solidify. The subgingival sample collected in the thioglycolate broth (Transport media) was mixed well by vortexing. The dilution of the sample was prepared using sterile saline solution in test tubes whereby 1 mL of the original sample was transferred into 9 mL of diluent (10^{-1} dilution) using a new sterile pipette.

2. Plating Method

Spread Plate Method: 0.1 ml of an appropriate dilution was pipetted onto the surface of a pre-prepared nutrient agar plate and spread evenly using a sterile swab. The plate was allowed to absorb the sample before incubating.

3. Incubation

The plates were inverted and incubated at 37°C for 24 hours.

4. Colony Counting and Calculation

After incubation, the visible colonies were counted on plates using a colony counter.

The study groups were as follows:

- Group 1: Full mouth scaling and root planing alone
- Group 2: Full mouth scaling and root planing + Antimicrobial Photodynamic Therapy with 0.5% Indocyanine Green Dye
- Group 3: Full mouth scaling and root planing + Antimicrobial Photodynamic Therapy with 1% Methylene Blue Dye

Colony forming units were assessed and counted at baseline, 1 week and 1 month interval for all the patients.

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III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; **Web**: www.klepkbsrc.org, **Phone**: 0831- 2444444, Extn. 4122

Result

Colony forming units were assessed and counted after test procedures at baseline, 1 week and 1 month interval.

At Baseline

COLONY FORMING UNIT – CFU/ml			
S.no	**Group 1**	**Group 2**	**Group 3**
1	1.23×10^2	1.25×10^2	1.21×10^2
2	1.43×10^2	1.41×10^2	1.42×10^2
3	1.56×10^2	1.54×10^2	1.56×10^2
4	1.78×10^2	1.72×10^2	1.76×10^2
5	1.25×10^2	1.21×10^2	1.24×10^2
6	1.27×10^2	1.25×10^2	1.23×10^2
7	1.56×10^2	1.54×10^2	1.55×10^2
8	1.98×10^2	1.98×10^2	1.97×10^2
9	1.77×10^2	1.72×10^2	1.75×10^2
10	1.34×10^2	1.43×10^2	1.4×10^2
11	1.53×10^2	1.51×10^2	1.51×10^2
12	1.23×10^2	1.26×10^2	1.21×10^2
13	1.26×10^2	1.22×10^2	1.2×10^2
14	1.29×10^2	1.27×10^2	1.26×10^2
15	1.45×10^2	1.43×10^2	1.4×10^2
16	1.45×10^2	1.43×10^2	1.43×10^2
17	1.37×10^2	1.37×10^2	1.38×10^2
18	1.78×10^2	1.76×10^2	1.79×10^2
19	1.76×10^2	1.78×10^2	1.8×10^2
20	1.23×10^2	1.25×10^2	1.27×10^2
21	1.25×10^2	1.23×10^2	1.25×10^2

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III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; **Web**: www.klepkbsrc.org, **Phone**: 0831- 2444444, Extn. 4122

At 1 Week Interval

COLONY FORMING UNIT – CFU/ml			
S.no	**Group 1**	**Group 2**	**Group 3**
1	1.03×10^2	0.78×10^2	0.82×10^2
2	1.2×10^2	0.93×10^2	1.01×10^2
3	1.32×10^2	0.98×10^2	1.14×10^2
4	1.43×10^2	1.2×10^2	1.31×10^2
5	1.02×10^2	0.73×10^2	0.83×10^2
6	1×10^2	0.78×10^2	0.83×10^2
7	1.31×10^2	0.98×10^2	1.11×10^2
8	1.69×10^2	1.38×10^2	1.53×10^2
9	1.47×10^2	1.17×10^2	1.29×10^2
10	1.12×10^2	0.93×10^2	0.98×10^2
11	1.26×10^2	0.95×10^2	1×10^2
12	1.01×10^2	0.8×10^2	0.8×10^2
13	1.03×10^2	0.75×10^2	0.8×10^2
14	1.03×10^2	0.82×10^2	0.85×10^2
15	1.18×10^2	0.95×10^2	0.97×10^2
16	1.21×10^2	0.95×10^2	0.97×10^2
17	1.12×10^2	0.89×10^2	0.95×10^2
18	1.49×10^2	1.2×10^2	1.31×10^2
19	1.52×10^2	1.23×10^2	1.33×10^2
20	1×10^2	0.75×10^2	0.85×10^2
21	1.03×10^2	0.74×10^2	0.85×10^2

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; **Web**: www.klepkbsrc.org, **Phone**: 0831- 2444444, Extn. 4122

At 1 Month Interval

COLONY FORMING UNIT – CFU/ml			
S.no	**Group 1**	**Group 2**	**Group 3**
1	0.71×10^2	0.42×10^2	0.55×10^2
2	0.92×10^2	0.55×10^2	0.75×10^2
3	0.98×10^2	0.57×10^2	0.78×10^2
4	1.12×10^2	0.81×10^2	0.91×10^2
5	0.75×10^2	0.39×10^2	0.55×10^2
6	0.78×10^2	0.37×10^2	0.57×10^2
7	0.98×10^2	0.58×10^2	0.88×10^2
8	1.21×10^2	0.83×10^2	1.01×10^2
9	1.07×10^2	0.77×10^2	0.95×10^2
10	0.86×10^2	0.59×10^2	0.7×10^2
11	0.91×10^2	0.51×10^2	0.81×10^2
12	0.71×10^2	0.4×10^2	0.56×10^2
13	0.75×10^2	0.36×10^2	0.59×10^2
14	0.78×10^2	0.43×10^2	0.59×10^2
15	0.89×10^2	0.57×10^2	0.68×10^2
16	0.91×10^2	0.67×10^2	0.71×10^2
17	0.87×10^2	0.57×10^2	0.63×10^2
18	1.09×10^2	0.8×10^2	0.97×10^2
19	1.19×10^2	0.81×10^2	1×10^2
20	0.71×10^2	0.38×10^2	0.56×10^2
21	0.75×10^2	0.37×10^2	0.57×10^2

The above-mentioned data has to be subjected to further statistical analysis.

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E-mail: research@kledeemeduniversity.edu.in; **Web**: www.klepkbsrc.org, **Phone**: 0831- 2444444, Extn. 4122

11/04/25

Ms. Sayali Manjarekar,
Laboratory Assistant,
Dr Prabhakar Kore Basic Science
Research Centre, KAHER, Belagavi.

12/4/25

Dr. Rubeen D. Nadaf
Scientist Grade I,
Dr Prabhakar Kore Basic Science
Research Centre, KAHER, Belagavi.

11/4/25

Dr. Suneel Dodamani,
Scientist Grade I,
Dr Prabhakar Kore Basic Science
Research Centre, KAHER, Belagavi.

15/4/2025

Dr. Ramesh S. Paranjape
Distinguished Professor, KAHER &
I/C Director,
Dr. Prabhakar Kore Basic Science
Research Centre, KAHER, Belagavi

ANNEXURE 3: PLAGIARISM CERTIFICATE

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences
A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State
Accredited 'A+' Grade by NAAC (3rd Cycle) Placed in Category 'A' by MHRD (GoI)
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Date : 15/4/2025 Serial No. : 409

PLAGIARISM CHECK REPORT

Name of the Applicant : **REG NO. IK0222002**
UG / PG / Ph.D / Staff : PG
Batch & Year : 2022
Department : Periodontics.

The soft copy of Research Work / Manuscript by **REG NO. IK0222002** KLEer. entitled
"Comparative evaluation of antimicrobial effect of indocyanin green & methylene blue dye as photosensitizers for photodynamic therapy as an adjunct to scaling & root Planing in Patients with Moderate Periodontitis - A RCT
under the guidance of . .....has been submitted for

Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of .....7.....%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

15/4/2025
Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

15/4/25
Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

ANNEXURE 4: BIOSTATISTICIAN CERTIFICATE

K L E
VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES
(Constituent College of K.L.E. University, Belgaum)
J.N.M.C. Campus, Nehru Nagar, Belgaum-590 010, Karnataka, India
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that Biostatistics aspect of the Dissertation/Research work of **REG NO. IK0222002** , **Post Graduate Student**, under the guidance of **Reader, Department of Periodontics**, entitled **"Comparative Evaluation of Antimicrobial Effect of Indocyanine Green and Methylene Blue as Photosensitizers for Photodynamic Therapy as an Adjunct to Scaling and Root Planing in Patients with Moderate Periodontitis – A Randomized Controlled Trial."** has been done under my guidance and completed satisfactorily.

Place: Belagavi
Date: 15-03-25

Name & Signature of Biostatistician

Dr. S. B. Javali. PH.D.
Professor in Statistics
Department of Community Medi...
JSM KLE International Medical ...
BELAGAVI-590...

ANNEXURE 5: INFORMED CONSENT

DEPARTMENT OF PERIODONTICS

KAHER'S KLE V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECT OF INDOCYANINE GREEN AND METHYLENE BLUE DYE AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN PATIENTS WITH MODERATE PERIODONTITIS - A RANDOMIZED CONTROLLED TRIAL

Principal Investigator: **REG NO. IK0222002**

I _____, aged _____ years have been informed about my involvement in the study.
I agree to give my personal details like Name, Age, Gender, Residential Address, Past and Present dental history, and any other details if required for the study to the best of my knowledge.
I will co-operate with the dentist.
I will follow the instructions given by the dentist during study.
I permit the dentist to utilize the information given by me and the results obtained from this study for presentation and publication without disclosing my identity.
I will visit the dentist as and when required for the study, at the given time and date.
I have understood the nature of the study and permit the dentist to perform the required radiographic, non-surgical, surgical and laser application procedures on me.
I will not claim any returns for co-operation in this study, even if it is being sponsored by any agency. I am participating with my own will and wish.
If for any reason I am unable to participate in the study, for reasons unknown, I can withdraw from the study.
In my full consciousness and presence of mind, after understanding all the procedures and related complications if any, in my vernacular language, I am willing and give my consent to participate in this study.

Date:

Name of the Patient:

Signature:

Address & Ph. No:

Name of witness/guardian:

Signature:

DEPARTMENT OF PERIODONTICS

KAHER'S KLE V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECT OF INDOCYANINE GREEN AND METHYLENE BLUE DYE AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN PATIENTS WITH CHRONIC PERIODONTITIS - A RANDOMIZED CONTROLLED TRIAL.

मुख्य अन्वेषक: **REG NO. IK0222002**

मी _____, वय _____ वर्षे अभ्यासातील माझ्या सहभागाबद्दल माहिती देण्यात आली आहे.
मी माझे वैयक्तिक तपशील जसे की नाव, वय, लिंग, निवासी पत्ता, भूतकाळ आणि वर्तमान दंत इतिहास आणि माझ्या माहितीनुसार अभ्यासासाठी आवश्यक असल्यास इतर कोणतेही तपशील देण्यास सहमत आहे.
मी दंतवैद्याला सहकार्य करीन.
अभ्यासादरम्यान दंतवैद्याने दिलेल्या सूचनांचे मी पालन करीन.
मी दंतचिकित्सकाला माझी ओळख उघड न करता सादरीकरण आणि प्रकाशनासाठी माझ्याद्वारे दिलेली माहिती आणि या अभ्यासातून मिळालेल्या निकालांचा वापर करण्याची परवानगी देतो.
दिलेल्या वेळी आणि तारखेला अभ्यासासाठी आवश्यक असेल तेव्हा मी दंतवैद्याला भेट देईन.
मला अभ्यासाचे स्वरूप समजले आहे आणि दंतवैद्याला माझ्यावर आवश्यक रेडियोग्राफिक, नॉन-सर्जिकल, सर्जिकल आणि लेझर ऍप्लिकेशन प्रक्रिया करण्यास परवानगी दिली आहे.
मी या अभ्यासात सहकार्यासाठी कोणत्याही परताव्याचा दावा करणार नाही, जरी ते कोणत्याही एजन्सीद्वारे प्रायोजित केले जात असले तरीही. मी माझ्या इच्छेने आणि इच्छेने सहभागी होत आहे.
कोणत्याही कारणास्तव मी अभ्यासात सहभागी होऊ शकलो नाही, अज्ञात कारणांमुळे, मी अभ्यासातून माघार घेऊ शकतो.
माझ्या पूर्ण जाणीवेने आणि मनाच्या उपस्थितीत, माझ्या स्थानिक भाषेत, सर्व प्रक्रिया आणि संबंधित गुंतागुंत समजल्यानंतर, मी या अभ्यासात सहभागी होण्यास तयार आहे आणि माझी संमती देतो.
तारीख:

पत्ता व दूरध्वनी क्रमांक:

स्वाक्षरी:

DEPARTMENT OF PERIODONTICS

KAHER'S KLE V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECT OF INDOCYANINE GREEN AND METHYLENE BLUE DYE AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN PATIENTS WITH MODERATE PERIODONTITIS - A RANDOMIZED CONTROLLED TRIAL

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ: **REG NO. IK0222002**

ನಾನು _____ ವಯಸ್ಸಿನ _____ ವರ್ಷಗಳ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಹೆಸರಿರುವ ಬಗ್ಗೆ ದೃಢೀಕರಿಸುತ್ತೇನೆ.

ನನ್ನ ವೈಯಕ್ತಿಕ ವಿವರಗಳಾದ ಹೆಸರು, ವಯಸ್ಸು, ರಿಂಗ್, ವಾಸಸ್ಥಳದ ವಿಳಾಸ, ಹಿಂದಿನ ಮತ್ತು ಪ್ರಸ್ತುತ ದಂತ ಇತಿಹಾಸ ಮತ್ತು ನನ್ನ ಜ್ಞಾನದ ಮಿತಿಗೆ ಅಧ್ಯಯನಕ್ಕೆ ಅಗತ್ಯವಿದ್ದರೆ ಇತರ ಯಾವುದೇ ವಿವರಗಳನ್ನು ನೀಡಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.

ನಾನು ದಂತವೈದ್ಯರೊಂದಿಗೆ ಸಹಕರಿಸುತ್ತೇನೆ.

ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ದಂತವೈದ್ಯರು ನೀಡಿದ ಸೂಚನೆಗಳನ್ನು ನಾನು ಅನುಸರಿಸುತ್ತೇನೆ.

ನನ್ನ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸದೆ ನಾನು ನೀಡಿದ ಮಾಹಿತಿಯನ್ನು ಮತ್ತು ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳನ್ನು ಪ್ರಸ್ತುತಿ ಮತ್ತು ಪ್ರಕಟಣೆಗಾಗಿ ಬಳಸಿಕೊಳ್ಳಲು ನಾನು ದಂತವೈದ್ಯರಿಗೆ ಅನುಮತಿ ನೀಡುತ್ತೇನೆ.

ನಾನು ದಂತವೈದ್ಯರನ್ನು ಅಧ್ಯಯನಕ್ಕೆ ಅಗತ್ಯವಿದ್ದಾಗ, ನಿರ್ದಿಷ್ಟ ಸಮಯ ಮತ್ತು ದಿನಾಂಕದಂದು ಭೇಟಿ ಮಾಡುತ್ತೇನೆ.

ನಾನು ಅಧ್ಯಯನದ ಸ್ವರೂಪವನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಮೇಲೆ ಅಗತ್ಯವಾದ ರೇಡಿಯೋಗ್ರಾಫಿಕ್, ಕನ್ಸರ್ವೇಟಿವ್ಸ್ಯುಲರ್, ಕನ್ಸರ್ವೇಟಿವ್ಸ್ ಮತ್ತು ಲೇಸರ್ ಅಪ್ಲಿಕೇಶನ್ ಕಾರ್ಯವಿಧಾನಗಳನ್ನು ನಿರ್ವಹಿಸಲು ದಂತವೈದ್ಯರಿಗೆ ಅನುಮತಿ ನೀಡಿದ್ದೇನೆ.

ಯಾವುದೇ ಏಜೆನ್ಸಿಯಿಂದ ಪ್ರಾಯೋಜಿತವಾಗಿದ್ದರೂ ಸಹ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಸಹಕಾರಕ್ಕಾಗಿ ನಾನು ಯಾವುದೇ ಆರ್ಥಿಕ ಪರಿಹಾರವನ್ನು ಕ್ಲೈಮ್ ಮಾಡುವುದಿಲ್ಲ. ನಾನು ನನ್ನ ಸ್ವಂತ ಇಚ್ಛೆ ಮತ್ತು ಆಯ್ಕೆಯಿಂದಲೇ ಭಾಗವಹಿಸುತ್ತಿದ್ದೇನೆ.

ಯಾವುದೇ ಕಾರಣಕ್ಕಾಗಿ ನನಗೆ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಾಧ್ಯವಾಗದಿದ್ದರೆ, ಅಜ್ಞಾತ ಕಾರಣಗಳಿಗಾಗಿ, ನಾನು ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಬಹುದು.

ನನ್ನ ಪೂರ್ಣ ದೃಷ್ಟಿ ಮತ್ತು ಮನಸ್ಸಿನ ಉಪಸ್ಥಿತಿಯಲ್ಲಿ, ಎಲ್ಲಾ ಕಾರ್ಯವಿಧಾನಗಳು ಮತ್ತು ಸಂಬಂಧಿತ ಹೊಣೆಗಾರಿಕೆಗಳು ಯಾವುದಾದರೂ ಇದ್ದರೆ, ನನ್ನ ಸ್ಥಳೀಯ ಭಾಷೆಯಲ್ಲಿ ಅರ್ಥಮಾಡಿಕೊಂಡ ನಂತರ, ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿರ್ಧರಿಸಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಒಪ್ಪಿಗೆಯನ್ನು ನೀಡುತ್ತೇನೆ.

ದಿನಾಂಕ:

ದಂತವೈದ್ಯ ಹೆಸರು: ಸಹಿ:

ರೋಗಿಯ ಹೆಸರು: ಸಹಿ:

ವಿಳಾಸ ಮತ್ತು ದೂರವಾಣಿ ಸಂಖ್ಯೆ.:

ಸಾಕ್ಷಿ / ಪೋಷಕರ ಹೆಸರು: ಸಹಿ

ANNEXURE 6: PROFORMA

DEPARTMENT OF PERIODONTICS

KAHER'S KLE V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI.

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFECT OF INDOCYANINE GREEN AND METHYLENE BLUE DYE AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN PATIENTS WITH MODERATE PERIODONTITIS - A RANDOMIZED CONTROLLED TRIAL

Case No: OPD No:

Name:

Age: Sex: Occupation:

Address:

Chief Complaint:

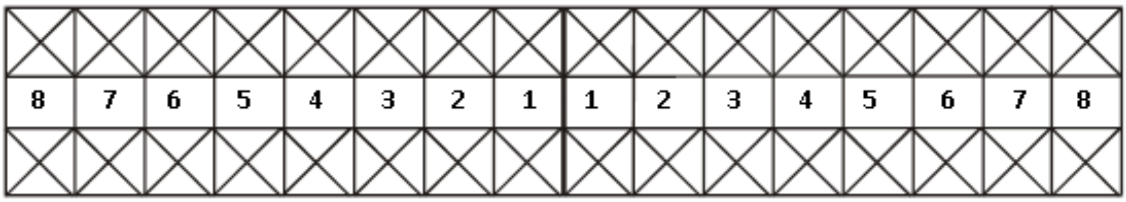
Medical History:

Dental history:

CLINICAL ASSESSMENT

Gingival Index (Loe and Silness)

At baseline

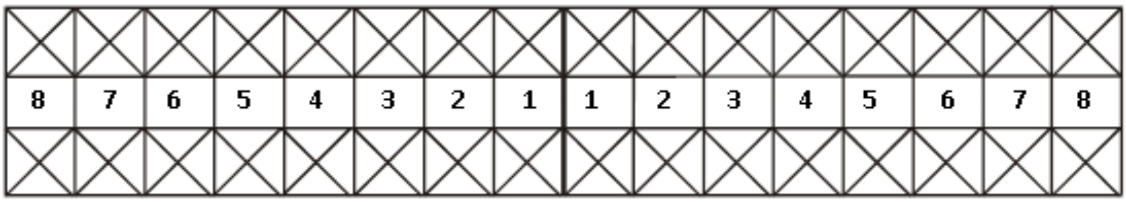


Total of mean score of all teeth =

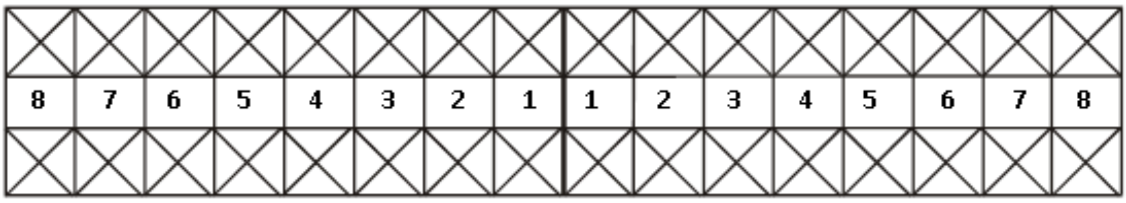
Total no. of teeth examined

GI Score =

At 7 days

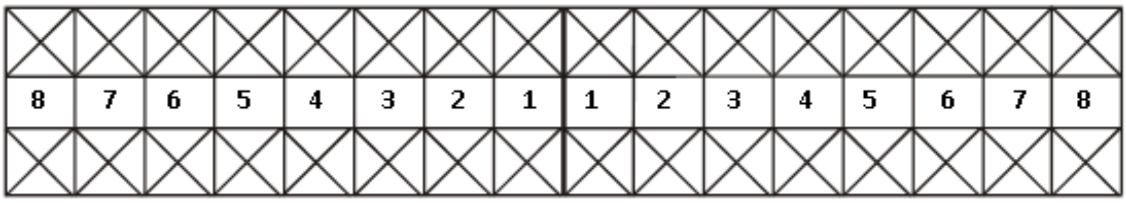


At 1 month

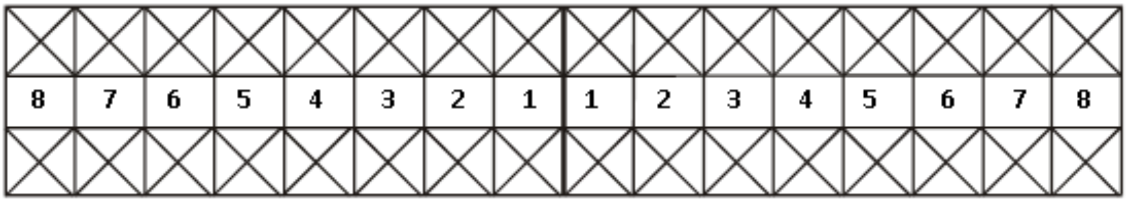


Pocket probing depth (PPD)

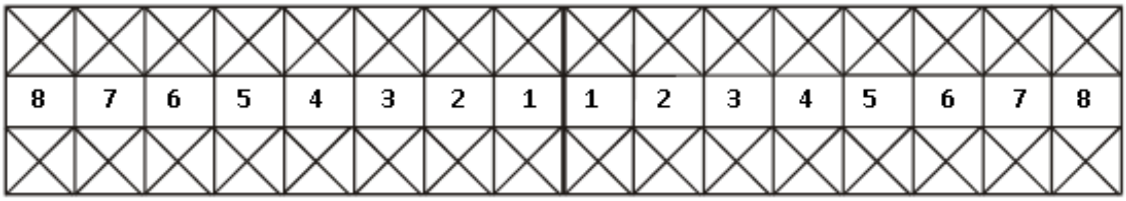
At baseline



At 7 days

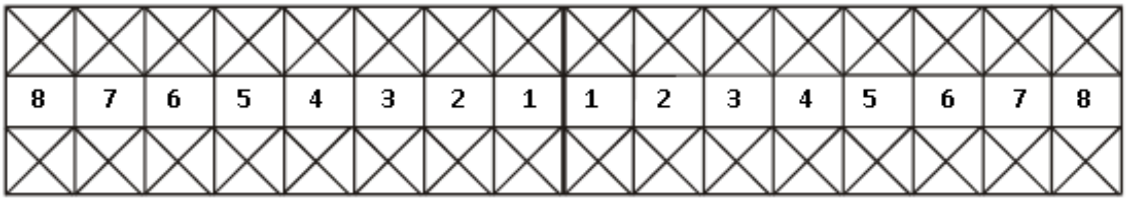


At 1 month

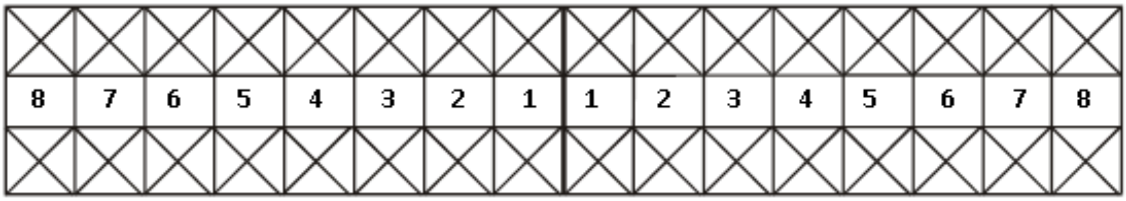


Clinical attachment loss (CAL)

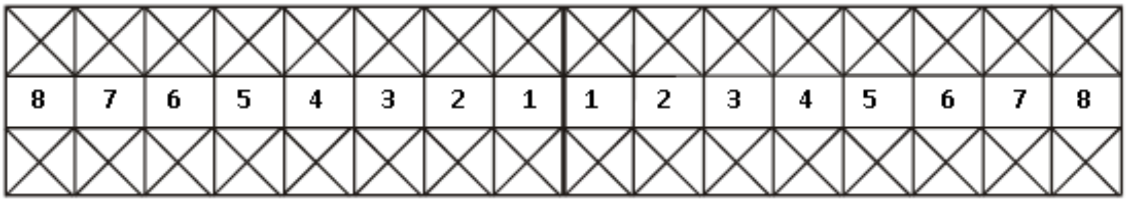
At baseline



At 7 days



At 1 month



MICROBIOLOGICAL ASSESSMENT

Number of colony forming units (CFU/ml)

BASELINE (CFU/ml)	
AT 7 DAYS (CFU/ml)	
AT 1 MONTH (CFU/ml)	

ANNEXURE 7: MASTER CHART

	Gingival index (GI)			Pocket Probing Depth (PPD)			Clinical Attachment Loss (CAL)		
Samples	**Baseline**	**1 week**	**1 month**	**Baseline**	**1 week**	**1 month**	**Baseline**	**1 week**	**1 month**
Group 1									
1	1.42	1.25	1.25	5	4	3	6	5	3
2	1.42	1.25	1.25	5	4	3	5	4	3
3	1.43	1.25	1.25	5	4	3	6	5	3
4	1.43	1.25	1.25	5	4	3	6	5	3
5	1.73	1.5	1.25	6	5	3	6	5	3
6	1.55	1.25	1.25	6	5	3	6	5	3
7	1.43	1.25	0.5	5	4	3	6	4	3
8	1.63	1.43	1.25	5	4	3	5	4	3
9	1.82	1.75	1.43	6	4	3	6	5	3
10	1.73	1.43	1.43	6	5	3	6	5	3
11	1.66	1.53	1.5	6	4	3	6	5	3
12	1.75	1.63	1.43	5	4	3	6	5	3
13	2.25	2.25	1.73	5	4	3	6	5	3
14	1.99	1.43	1.25	5	3	3	6	4	3
15	1.83	1.53	1.25	5	4	3	5	4	3
16	1.63	1.54	1.43	6	5	3	6	5	3
17	1.74	1.43	1.43	5	4	3	6	4	3
18	2.5	2.5	1.75	5	4	3	6	4	3
19	1.42	1.25	0.5	6	5	3	6	5	3
20	2.25	2.25	1.43	6	4	3	6	4	3
21	2.25	1.73	1.25	5	4	3	5	4	3
Group 2									
1	1.88	1.43	1.25	6	3	2	6	3	2
2	1.74	1.23	0.5	5	2	2	5	2	2
3	1.66	1.23	0.75	5	2	2	5	2	2
4	1.97	1.2	0.5	5	2	2	5	2	2
5	2.23	1.43	0.5	6	3	2	6	3	3
6	1.73	0.75	0.25	6	3	2	6	3	2
7	1.62	0.25	0.25	5	2	2	5	2	2
8	1.66	1.23	0.34	5	2	2	5	2	2
9	2.23	1.25	0.34	6	3	3	6	3	3
10	1.75	0.75	0.25	6	3	2	6	3	3
11	1.76	0.75	0.25	5	3	3	5	3	2
12	1.43	1.24	0.25	5	3	3	5	3	2
13	1.74	0.5	0.34	5	2	2	5	2	2
14	1.43	0.5	0.25	6	3	2	6	3	3
15	1.74	1.25	0.5	6	3	3	6	3	2
16	1.56	0.55	0.25	6	3	2	6	3	3

17	1.42	0.55	0.25	6	3	3	6	3	3
18	1.42	0.55	0.34	5	3	2	5	3	3
19	1.73	0.23	0.23	5	2	2	5	2	2
20	1.55	0.75	0.5	5	2	2	5	2	3
21	1.68	1.25	0.5	6	3	2	6	3	2
Group 3									
1	1.55	1.25	0.23	5	3	3	5	3	3
2	1.63	1.23	0.75	6	3	3	6	3	3
3	1.99	1.53	0.5	5	2	2	6	3	2
4	1.83	1.5	0.5	6	4	3	6	4	3
5	1.63	1.25	1.25	5	3	3	5	3	3
6	1.74	1.5	0.34	5	2	2	5	2	2
7	2.5	1.5	0.34	6	3	3	6	3	3
8	1.42	1.25	0.25	6	4	3	6	4	3
9	2.25	1.5	0.5	5	3	3	5	3	3
10	1.88	0.75	0.34	5	3	3	5	3	3
11	1.66	0.25	0.25	6	3	3	6	3	3
12	1.97	0.75	0.75	6	3	3	6	3	3
13	2.27	1.25	0.34	6	4	3	6	4	3
14	1.43	0.5	0.25	5	3	3	5	3	3
15	1.74	0.75	0.34	5	3	2	6	4	3
16	1.56	0.5	0.25	5	3	2	5	3	2
17	1.56	0.5	0.5	6	3	3	6	3	3
18	1.43	0.5	0.5	5	3	3	5	3	3
19	1.42	1.25	0.75	6	3	3	6	3	3
20	1.72	1.25	0.5	5	3	2	5	3	3
21	1.56	0.75	0.25	6	3	3	6	3	3

	Colony forming units (CFU/ml)		
Samples	**Baseline**	**1 week**	**1 month**
Group 1			
1	1.23	1.03	0.71
2	1.43	1.2	0.92
3	1.56	1.32	0.98
4	1.78	1.43	1.12
5	1.25	1.02	0.75
6	1.27	1	0.78
7	1.56	1.31	0.98
8	1.98	1.69	1.21
9	1.77	1.47	1.07
10	1.34	1.12	0.86
11	1.53	1.26	0.91
12	1.23	1.01	0.71
13	1.26	1.03	0.75
14	1.29	1.03	0.78
15	1.45	1.18	0.89
16	1.45	1.21	0.91
17	1.37	1.12	0.87
18	1.78	1.49	1.09
19	1.76	1.52	1.19
20	1.23	1	0.71
21	1.25	1.03	0.75
Group 2			
1	1.25	0.78	0.42
2	1.41	0.93	0.55
3	1.54	0.98	0.57
4	1.72	1.2	0.81
5	1.21	0.73	0.39
6	1.25	0.78	0.37
7	1.54	0.98	0.58
8	1.98	1.38	0.83
9	1.72	1.17	0.77
10	1.43	0.93	0.59
11	1.51	0.95	0.51
12	1.26	0.8	0.4
13	1.22	0.75	0.36
14	1.27	0.82	0.43
15	1.43	0.95	0.57
16	1.43	0.95	0.67
17	1.37	0.89	0.57
18	1.76	1.2	0.8
19	1.78	1.23	0.81
20	1.25	0.75	0.38
21	1.23	0.74	0.37

Group 3			
1	1.21	0.82	0.55
2	1.42	1.01	0.75
3	1.56	1.14	0.78
4	1.76	1.31	0.91
5	1.24	0.83	0.55
6	1.23	0.83	0.57
7	1.55	1.11	0.88
8	1.97	1.53	1.01
9	1.75	1.29	0.95
10	1.4	0.98	0.7
11	1.51	1	0.81
12	1.21	0.8	0.56
13	1.2	0.8	0.59
14	1.26	0.85	0.59
15	1.4	0.97	0.68
16	1.43	0.97	0.71
17	1.38	0.95	0.63
18	1.79	1.31	0.97
19	1.8	1.33	1
20	1.27	0.85	0.56
21	1.25	0.85	0.57