"EVALUATION AND COMPARISON OF THE EFFECT OF INDOCYANINE GREEN, PHOTOTHERMAL THERAPY AND PHOTODYNAMIC THERAPY ON TITANIUM ADHERENT BIOFILM OF PORPHYROMONAS GINGIVALIS - AN IN VITRO STUDY"

By

REG NO. IK0220004

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This is to certify that the dissertation ***"Evaluation and Comparison of the effect of Indocyanine green, Photothermal therapy and Photodynamic therapy on Titanium adherent biofilm of Porphyromonas gingivalis - an in vitro study"*** *is a bonafide research work done by* ***REG NO. IK0220004.***

Dr. VINAYAK KUMBHOJKAR *M.D.S*
Professor & Head,
Department of Periodontics,
KAHER's KLE V. K. Institute of
Dental Sciences, Belagavi
Professor and Head
Department of Periodontics
KLE V. K. Institute of Dental Sciences
Belagavi
Date: 29/11/22
Place: Belagavi

Dr. ALKA KALE *M.D.S*
Principal,
KAHER's KLE V. K. Institute of
Dental Sciences, Belagavi
PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.
Date: 29/11/22
Place: Belagavi

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I, **REG NO. IK0220004**, a postgraduate student in Dept of Periodontics, have completed my research work on the topic **"***Evaluation and Comparison of the effect of Indocyanine green, Photothermal therapy and Photodynamic therapy on Titanium adherent biofilm of Porphyromonas gingivalis - an in vitro study***"** in the year 2020-2023.

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Date:

Place: Belagavi **REG NO. IK0220004**

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LIST OF ABBREVIATIONS

ICG	Indocyanine green
PTT	Photothermal therapy
PDT	Photodynamic therapy
MB	Methylene Blue
TBO	Toluidine blue O
ANOVA	Analysis of variance
IL	Interleukin
ml	Milliliter
µl	Microliter

ABSTRACT

INTRODUCTION:

Peri-implantitis is an oral disease that is known to cause inflammation around Osseointegrated implants and their supporting structures due to the presence of plaque (dental biofilm). It is caused by the accumulation of dental plaque which is a structurally and functionally well-organized biofilm that normally maintains a homeostatic relationship with the human host. A disturbance in this balance causes a microbial shift from commensal to pathogenic periodontal pathogens that mark the beginning of the peri-implant disease. The frontline treatment for peri-implant diseases includes scaling and root planning (SRP) that effectively removes the plaque and restores it to a healthy state and the use of antimicrobial agents such as Chlorhexidine that are often used as an adjunct to SRP to aid and maintain the healthy state of tissues. However, these antimicrobial agents have side effects such as alteration of taste, discoloration of teeth, and development of antimicrobial resistance. The conventional treatment doesn’t eliminate the biofilm due to its complex structure. Hence, there has been a shift in research towards new non-invasive technique as LASER. However, the laser may cause some detrimental thermal effects on the surrounding periodontal tissues, leading to potential and unexpected side effects. Recently, an alternative approach named antimicrobial photodynamic therapy (PDT) has been developed for the decontamination of implant surfaces. The application of PDT in dentistry is growing rapidly. The purpose of the current study is to assess and compare the antimicrobial activity of Indocyanine green, Photothermal therapy, and Photodynamic therapy on the titanium adherent biofilm of *Porphyromonas gingivalis.*

AIM:

To assess and compare the antimicrobial activity of Indocyanine green, Photothermal therapy and Photodynamic therapy on titanium adherent biofilm of *Porphyromonas gingivalis.*

MATERIALS AND METHODS:

This is an experimental in-vitro microbial study. 120 pre-sterilized titanium of 8mm and thickness of 2mm was obtained and the disc was inoculated with a strain of *porphyromonas gingivalis* and kept in an anaerobic chamber for 48 hours. The inoculated disc was randomly allocated into four groups. Group 1: control group; group 2: photosensitizer (Test group 1); group 3: Photothermal therapy (Test group 2); group 4: Photodynamic therapy (Test group 3). The dye used was Indocyanine green dye. Diode laser was used with 940 nm at 0.1 power watt at 5J/cm^2 for 30-40 sec. The data were entered in Excel and analyzed statistically using the SPSS software version. Intergroup comparisons were done by One-way ANOVA. A pairwise Comparison of four groups was also carried out using an LSD post hoc test. All statistical tests were performed at a significance level of 5% ($p<0.05$).

RESULTS AND CONCLUSION:

In test groups, there was a significant reduction in *Porphyromonas gingivalis* colony count in ICG, PTT, and PDT groups. The maximum reduction in *Porphyromonas gingivalis* bacterial colony count was noted with PDT (Mean ±SD is $20.7 \pm 2.7 \times 10^4$) while the minimum reduction was noted in the ICG group (Mean±SD is $51 \pm 2.6 \times 10^4$). Photothermal therapy also showed a reduction in *Porphyromonas gingivalis* bacterial colony count with $34.3 \pm 4.2 \times 10^4$) Therefore, it is described that

Photodynamic therapy (PDT) showed maximum reduction in *Porphyromonas gingivalis* bacterial colony count when compared to other treatments protocols and shows there is a statistically significant result of *P value* <0.001

KEYWORDS: Implant, Indocyanine green, Laser, Peri-implantitis, Photodynamic therapy.

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INTRODUCTION

Dental implants have become an established therapy in dentistry to replace missing teeth in different clinical situations. The success rate of dental implants has been reported as 82.9% in 16 years study[1]. It considered as a “safe” treatment option by following proper care and attention of anatomical and intra-individual limiting factors during insertion of dental implants. Nevertheless, in the last decades, one of the most frequent complications that has been raised is the presence of peri-implant inflammations affecting both the surrounding soft and hard tissues that can lead to the loss of the implant.

Peri-implantitis is an inflammatory process that affects the tissues around an Osseointegrated implant and results in the loss of supporting bone beyond initial biologic bone remodeling around an implant in function.[2,3] The prevalence of peri-implantitis was reported from less than 7% to 37% of implants. The variation of prevalence can be associated with differences in studied populations, follow-up time, implant variables, and the criteria that had been used to define peri-implantitis.[4,5] Peri-implantitis exhibit similar microbial flora as chronic periodontitis. Although there is no consensus regarding microorganisms, the systematic review done by Perez-Chaparro and colleagues[6] identified three commonly occurring periodontal pathogens associated with peri-implantitis: *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*. The dental implant also exhibits all the signs of peri-implant diseases including exudation, increased pocket depths, and crater-like osseous defects, that are strictly localized around the dental implant. If it is left untreated, there will be significant bone loss, infection, and mobility that could leads to loss of implant osseointegration. Other predisposing factors of peri-implantitis include the presence of aggressive bacteria, excessive mechanical stress, and corrosion. These factor that could act synergistically with biofilm to worsen the condition. Therefore, oral microflora is considered as the defining factor for the success or the failure of a dental implant.

Conventional management principally involves eliminating the causative pathogens and, in doing so, arresting the inflammatory response. Various treatment modalities include mechanical debridement such as dental curettes, ultrasonic scalers, and air-powder abrasive and chemical like citric acid, H_2O_2, chlorhexidine digluconate, and EDTA procedures[6,7] and laser therapy. And the basic treatment for periodontitis and peri-implantitis is mechanical debridement. Decontamination of an implant surfaces is a challenging goal. Because the screw-shaped design of the implants, combined with various surface modifications of titanium, may facilitate plaque accumulation, resulting in formation of bacterial biofilm. Therefore, the initial stage of treatment includes the elimination of plaque and calculus, decontamination of the implant surface, and maintenance of healthy conditions of tissues.[8] On such surfaces, mechanical debridement may have a limited effect and certainly does not result in the complete removal of all adhering microorganisms. Given that mechanical debridement alone cannot eliminate all pathogens, due to factors such as inaccessible reservoirs of bacteria in the deep periodontal tissue and the supporting structures. To overcome these limitations of conventional mechanical therapy, various antimicrobial or antiseptic agents are used as an adjunctive to conventional therapy for treatment of periodontitis or peri-implantitis. However, due to the development bacterial resistance, the efficacy of these adjunctive is also limited for periodontal treatment [9.] As a result, there is a need for development of alternative antimicrobial approaches for periodontal treatment. Over the years, a novel non-invasive laser therapy had been proposed to improve the non-surgical treatment options of peri-implantitis.

The laser has been proposed as a new technical modality in the treatment of periodontal diseases. Different types of lasers are used as an effective means of decontamination of periodontal pockets over a period of years. The most frequently used laser includes a diode, erbium lasers, and CO_2 due to their hemostatic properties, selective calculus ablation, and bactericidal effects. Lasers are known to kill bacteria due to their high bactericidal properties, and they have demonstrated effective killing of oral pathogenic bacteria associated with periodontitis and peri-implantitis[10].In a recent literature review, they summarized that diode lasers are able to kill bacteria through photo-thermal effects. Furthermore studies, have shown that low-level diode lasers are also able to inactivate the bacterial endotoxins such as lipopolysaccharides of Gram-negative bacteria involved in peri-implantitis. The diode laser with a wavelength of 940 nm used in the present study has the ability to decontaminate the implant surface.

In recent years, several authors have described that the antimicrobial activity and efficacy of laser light, depends on its photothermic effects, in both in vitro and in vivo studies.[11-14] Microbiological studies in periodontology have shown that diode laser can significantly reduce the periodontopathogenic bacteria such as *Porphyromonas gingivalis, Prevotella intermedia, and Aggregatibacter actinomycetemcomitans* (formerly Actinobacillus actinomycetemcomitans) with lethal photosensitization.[15,16] However, lasers may cause some detrimental thermal effects on the surrounding periodontal tissues leading to potential and unexpected side effects. As a result, there is a need for development of alternative antimicrobial approaches for periodontal treatment.

An alternative approach to decontamination of dental implant surface is antimicrobial photodynamic therapy (aPDT). The mechanism behind antimicrobial photodynamic therapy (aPDT) is the use of a low-power laser in combination with photosensitizing compounds. The components of photosensitizing compounds are linked to the bacterial membrane and, when excited, react with the substrate. The photosensitizer binds to the target infected cells and when it is irradiated with light of specific wavelength, in the presence of oxygen, it undergoes a transition from a low-energy ground state to an excited singlet state; then singlet oxygen and other very reactive agents are produced, which are toxic to targeted infected cells.[17]

Typically, an aPDT has been assessed in the context of conventional photosensitising agents, such as toluidine blue and methylene blue. These conventional agents act through photochemical means and appear to be of limited clinical benefit[18-20]. However, contemporary photosensitising agents have now been developed, with the promise of greater efficacy in the management of periodontal disease. One such photosensitising agent that is commonly investigated is indocyanine green (ICG). This anionic photosensitiser has a peak absorption higher than conventional agents and displays its effects primarily through photothermal activity, which is in contrast to the conventional agents that exert their effects through photochemical means [21-23].

Moreover, ICG has in vitro shown to be taken up significant result in periodontal pathogens, namely, *Aggregatibacter actinomycetemcomitans*, and is therefore highly efficacious in eliminating microorganisms highly associated with periodontitis.Furthermore, ICG mediated photodynamic therapy (ICG-PDT) is effective in eliminating antimicrobial-resistant strains of commonly occurring bacterial species. Collectively, this evidence indicates that ICG-PDT may provide clinical benefits in scenarios which are outside the remit of conventional antimicrobials[23].

However, there is no overall consensus on the clinical benefits of ICG-PDT, and therefore, robust synthesis of the literature evaluating ICG – PDT is required in order to establish whether it may be beneficial for the management of periodontal disease, and current evidence is based solely upon outdated conventional photosensitizing agents. Thus, the aim of this in-vitro study is to compare the efficacy of photothermal therapy, indocyanine green and antimicrobial photodynamic therapy against periodontal pathogen namely *Porphyromonas gingivalis* that is adherent on titanium disc.

AIM AND OBJECTIVES

AIM OF THE STUDY:

- To access and compare the effect of Indocyanine green, photothermal therapy and photodynamic therapy on titanium adherent biofilm of *Porphyromonas gingivalis.*

OBJECTIVES OF THE STUDY:

- To access the effect of photosensitizer (Indocyanine-green) on titanium coated biofilm of *Porphyromonas gingivalis.*

- To access the effect of Photothermal therapy (Diode laser) on titanium coated biofilm of *Porphyromonas gingivalis.*

- To access the effect of Photodynamic therapy (indocyanine green + Diode laser) on titanium coated biofilm of *Porphyromonas gingivalis.*

- To compare the effect of the photosensitizer, photothermal therapy and photodynamic therapy on titanium coated biofilm of *Porphyromonas gingivalis*

REVIEW OF LITERATURE

The goal of modern dentistry is to restore the patient to normal function, speech, health and aesthetics. The evolution in the modern age of dental implantology began with the introduction of a two-stage threaded titanium root-form implant. Dr P Branemark who is known as the father of modern implantology developed and tested a system using pure titanium screws which he termed fixtures. These were first placed in 1965 and were the first to be well-documented and the most well-maintained dental implant so far.[5]

Currently, commercially pure titanium and Ti-Al-V alloy have become the gold standard in implant dentistry, although ceramic materials with the use of zirconium dioxide and innovative metallic alloys are attracting interest in implantology.[6] Nevertheless, in the last decades, one of the most frequent complications has been raised is the presence of peri-implant inflammations affecting both the surrounding soft and hard tissues that can lead to the loss of the implant. Therefore, in dentistry strategies should be integrated in modern rehabilitation concepts for prevention and treatment of peri-implant disease.

Over the years, the use of the laser in dentistry has been increased and improved. Laser irradiation exhibits strong ablation, haemostasis, detoxification and bactericidal effects on the human body. These effects can be useful during periodontal treatment, especially for handling of the soft tissue as well as for the debridement of diseased tissues. The laser application in the periodontal treatment is a part of a non-surgical and act as adjunct to surgical approaches, is used for periodontal pockets decontamination due to its bactericidal effect, and the removal of granulation tissues, inflamed and diseased epithelium lining, bacterial deposits and subgingival calculus. Thus, laser therapy serves as an alternative or adjunctive therapy to mechanical approaches, in periodontal therapy. Each laser has different characteristics because of their different wavelengths. Thus, the operator must be aware of the possible risks involved in clinical applications, and precaution must be exercised to minimize these risks when performing laser therapy. However, lasers may cause some detrimental thermal effects on the surrounding periodontal tissues leading to potential and unexpected side effects.

As a result, there is a need for development of alternative antimicrobial approaches for periodontal treatment. Recent advances in treating periodontal and peri-implant diseases are antimicrobial photodynamic therapy (aPDT) which is a two-stage treatment that combines light energy with a drug (photosensitizer) designed to destroy cancerous and precancerous cells after light activation. Photosensitizers are activated by a specific wavelength of light energy, usually from a laser. The photosensitizer is nontoxic until it is activated by light. However, after light activation, the photosensitizer becomes toxic to the targeted tissue.

Several photosensitizer drugs are available today to treat a variety of diseases, including acne, psoriasis, age-related macular degeneration, and several cancers, such as skin, lung, brain, bladder, pancreas, bile duct, esophagus, and head and neck.

In addition to treating these conditions, PDT also helps treat bacterial, fungal and viral infections. Studies have shown that this light-based therapy can trigger the body's immune response, giving your body another means to help destroy infected cells and tissues.

1. **Azizi B et al (2019);** In this study the author had randomly divided the implants into four experimental groups and two control groups (n = 12 each), the following treatment protocols followed in this study include -Group 1 (PDT1)-PDT with toluidine blue; Group 2 (PDT2)-PDT with phenothiazine chloride dye; Group 3 (LAD)-light emitting diode (LED) with toluidine blue; Group 4 (TB)-treatment with only toluidine blue. In the positive control (PC) group, the implants were treated with a 0.2% chlorhexidine- based solution, and in the negative control (NC) group, no treatment was used. From this study they concluded that there is a highest bacterial reduction were recorded in the PDT1 (PDT with toluidine) - 98.3% and PDT2 (PDT with phenothiazine chloride dye) - 97.8% groups both having statistically significant reduction compared to Negative Control (NC) group (<0.05). Light-activated device was less effective than PDT1 and PDT2, without statistically significant difference compared with NC or any other treatment group and from this study, they considered this antimicrobial photodynamic therapy as a successful alternative treatment option for decontaminating titanium dental implants. This effective treatment protocol should be established for decontaminating implant surfaces and should be further investigated in clinical studies.

2. **Ghasemi M et al (2019);** In this study, they investigated the effect of aPDT with laser or light emitting diode (LED) compared with conventional chlorhexidine treatment on the titanium-attached biofilms of Aggregatibacter actinomycetemcomitans. They used Thirty-six acid-etched and sandblasted (SLA) titanium discs and were allocated to six groups and the disc were incubated with the titanium-adherent biofilms of A. actinomycetemcomitans.

A negative control is no treatment has done, positive control group they used 0.2% chlorhexidine solution, TBO group contains of 0.1 mg/mL Toluidine Blue [TBO], PDT group includes aPDT-treated subjected either with diode laser with a wavelength of 635 nm wavelength or LED with the peak wavelength of 630 nm with TBO as photosensitizer and sterile control (not contaminated). And at last number of colony-forming units (CFUs) per disc were calculated. From this study concluded that both Light activated devices and diode lasers have a lower ability to suppress *A. actinomycetemcomitans* biofilms compared to 0.2% chlorhexidine in vitro. However, the photodynamic therapy with the use of LED as a light source and Toluidine Blue-O as a photosensitive agent could be an appropriate alternative to conventional chlorhexidine treatment.

3. **Smeo K et al (2018);** In this study, the author conducted a descriptive analysis by reviewing both in vivo and in vitro studies mainly concerned about the anti-bacterial effect of diode lasers (810nm, 940nm, 980nm) on implant surfaces at various parameters for peri-implant therapy. In this review author search was limited to 10 years and mainly focused on PubMed and Google Scholar from January 2007- March 2017). The author concluded that the Diode laser is an effective adjunctive tool in the treatment of peri-implantitis in combination with mechanical therapy and it doesn't have any negative effect on surrounding soft and hard tissues on implant surfaces and concluded that 810 nm wavelength with low average power and appropriate irradiation time to treat the peri-implantitis had given significant result without affecting or altering the surrounding tissues and it ensures the complete removal of

inflammatory signs such as Bleeding on probing , pocket depth and suppuration, whereas a conventional therapy cannot eliminate or decontaminate bacteria completely and effectively alone. Complete or almost complete elimination of bacteria from the implant surface needed for treatment of peri-implantitis with-out increase of temperature or surface characteristic changes of the implant surfaces according to the reviewed vivo and vitro studies can be achieved by using a diode laser 810 nm as adjunctive tool and in combination with a conventional therapy with a power not more 1 W in continuous wave mode for 20 s five times with 30-s pause (after each 20-s application time) or pulse wave mode for 100-ms pulse duration to ensure completely removal of inflammatory signs such as BOP, PD, suppuration, and bone loss, whereas a conventional therapy can not eliminate a bacteria completely and effectively alone he present study aims to conduct a descriptive analysis by reviewing in vivo and in vitro studies concerned with the antibacterial effect of diode lasers (810 nm, 940 nm, and 980 nm) and their effects on implant surfaces at different parameters for peri-implantitis treatment. Materials and methods The PubMed and Google Scholar had been used to search for articles focused on the antibacterial effect of diode lasers (810 nm, 940 nm, and 980 nm) in the treatment of peri-implantitis and their effects on implant surfaces. This literature search was limited to 10 years (January 2007–March 2017). Results Diode laser is an effective adjunctive tool in treatment of peri-implantitis in combination with a conventional therapy without any negative effect on the surrounding soft and hard tissues, and on the implant surfaces where the favorable settings regarding to vivo and vitro studies are 810 nm in non-contact continuous wave mode at 1 W for 20 s, five times with 30-s pause after each 20-s application time, and 600-µm fiber tip or 810 nm in non-contact pulse wave mode at 1 W, 50 Hz and a pulse duration of 100 ms/pulse, 30-s application time (2 times for each side). Conclusion Eight hundred ten-nanometer diode laser wavelength with a low average power and appropriate irradiation time treat the peri-implantitis significantly without affecting the surrounding tissues or alteration of the implant surface

4. **Azizi B et al (2018);** In this study, the author evaluated the antimicrobial efficacy of photodynamic therapy (PDT) and light-activated disinfection (LAD) on zirconia dental implants contaminated with three bacterial species and investigated PDT and LAD and had seen if there are any alterations in implant surface. In this study, they have taken zirconia dental implants (n=72) and incubated them with three bacterial species that includes *Prevotella intermedia, Actinomyces actinomycetemcomitans, and Porphyromonas gingivalis.* According to the decontamination protocal the implants were randomly divided into four groups (n=12 dental implants/each). The groups include - Group 1 (PDT1) - PDT (660 nm, 100 mW) with toluidine blue; Group 2 (PDT2) - PDT (660 nm, 100 mW) with phenothiazine chloride dye; Group 3 (LAD) - light emitting diode (LED) with toluidine blue; and Group 4 (TB) - toluidine blue without the application of light. positive control (PC) group implants were treated with a 0.2% chlorhexidine-based solution, and those implants assigned to negative control (NC) group did not undergo any treatment. After 72 hours, the colony forming units (CFU) were counted. Distinctive colonies were confirmed with MALDI Biotyper. The implants

were analyzed using scanning electron microscope (SEM) to evaluate the possible surface alterations due to PDT or LAD. All study groups had significant reductions in the number of CFUs compared with the Negative control (NC). PDT1(TBO) (98.3%), the PDT2(Phenothiazine chlorine dye) (97.8%), and the LAD groups had the largest bacterial reduction with respect to each bacterial species separately and the total bacterial count, and they were more efficient compared with the TB group. They concluded that both PDT and LAD showed had high and equal effectiveness in decontamination of zirconia dental implants.

5. **Birang E et al (2019);** In this in-vivo study, the contaminated discs, except for the negative control group, other discs were randomly undergone one of five treatments that includes Erbium: Yttrium Aluminum Garnet (Er-YAG) laser, plastic curette, 0.12% chlorhexidine, PDT, and 810nm diode laser. A spectrophotometer was used to measure Optical Density (OD) in the case of aerobic microorganisms. Colony-Forming Units (CFUs) were used for anaerobic bacteria. And they investigated the effect of study methods on anaerobic bacteria after 48 hours, and the results showed a significant difference among 6 groups in terms of CFUs ($P < 0.001$). From this study the author concluded that all five mechanicals (plastic curette), chemical (CHX), laser (810nm diode and Er: YAG), and aPDT methods could reduce oral biofilms from roughed surfaces of titanium discs. But comparing all five treatments Er: YAG laser had the highest effects and plastic curette had the lowest effects.

6. **Birang E et al (2017);** In this randomized clinical trial, the author evaluated the effectiveness of photodynamic therapy with low-level diode laser in 40 implants presenting primary peri-implantitis in 20 patients. Before starting with laser and photodynamic therapy, conventional treatment comprising scaling and root planing (SRP) was accomplished for the whole mouth by mechanical debridement with titanium curettes and air polishing with sodium bicarbonate powder was accomplished around the implants. And then implants were randomly divided into two groups and treated with LT (control) and PDT (test). The clinical indices were measured at baseline, 6 weeks and 3 months after treatment. Real-time polymerase chain reaction (PCR) was used for analysis of microbial samples at baseline and 3-month follow-up. And they concluded that both groups showed statistically significant improvements in terms of bleeding on probing (P0.05). The number of Aggregatibacter actinomycetemcomitans (P=0.022), Tannerella forsythia (P=0.038) and Porphyromonas gingivalis (P=0.05) in the test group and Porphyromonas gingivalis (P=0.015) in the control group significantly decreased. But the author suggested that both LT and PDT have significant short-term benefits in the treatment of primary peri-implantitis.

7. **Saffarpour A et al (2016);** In this study ,the author had used fifty SLA implants wivided into five groups and were incubated with *A actinomycetemcomitans* bacteria to form bacterial biofilm. The groups included in this study were Group 1 underwent Er:YAG laser radiation (with 10-Hz frequency, 100-mJ energy, and 1-W power); group 2 was subjected to LED (with 630-nm wavelength and maximum output intensity of 2.000 to 4.000 mW/cm2) and TBO as a photosensitizer; group 3 was exposed to diode

laser radiation (with 810-nm wavelength and 300-mW power) and ICG-based PS; and group 4 was immersed in 2% CHX. Group 5 was the control group, and the samples were rinsed with normal saline. The number of colony-forming units (CFU) per implant was then calculated. Data were analyzed and the five groups were compared and they concluded that there is a significant difference was found between the control group and the other groups (P < .01). The lowest mean of CFU per implant count was in group 4 (P < .01), and the highest mean belonged to the control group. Photodynamic therapy by TBO + LED and ICG-based PS + diode laser was more effective than Er:YAG laser irradiation in suppression of this organism (P < .01). There was no significant difference between groups 2 and 3. Fi,nally the author concluded the antibacterial effect of 2% CHX was greater than that of other treatment methods that were compared in this study.

8. **Bouhout Y et al (2015);** In this review of literature, the author aim is to review the efficacy of erbium: Yttrium-aluminum-garnet and neodymium: Yttrium-aluminium-garnet laser treatment could either replace or complete conventional mechanical/surgical periodontal treatments because over the years, the use of laser in dentistry had been increased and improved. In periodontal and peri-implant treatment the application of laser takes part as a non-surgical approaches or adjunct to the surgical approach for decontamination of periodontal pockets due to its bactericidal effect and it helps in removal of granulation tissues, inflamed and diseased epithelial lining, and bacterial deposits and subgingival calculus. In spite of its beneficial effect, the capacity of laser to replace the conventional treatment for chronic

periodontitis is still debatable. In this review the author concluded that the laser therapy could be replace or even add on to our conventional periodontal treatment is still doubtful. So future studies need to determine laser effectiveness for root scaling and planning, removal of calculus, decontamination of bacteria and specially, many randomized clinical trials should be performed by independent researchers in order to demonstrated the real role of lasers in the management of periodontal and peri-implant patients.

9. **Roncati M et al (2013);** In this case report, the author had used an 810-nm diode laser to treat non-surgically 7-mm pocket around an implant that had five threads of bone loss, Bleeding on Probing, and exudate, and the patient was followed up for 5 years. Non-surgical treatment, home care reinforcement, clinical indices records, and radiographic examination were completed in two consecutive 1-h appointments within 24 h. The patient was monitored frequently for the first 3 months. Subsequent, maintenance debridement visits were scheduled at 3-month intervals. By this treatment the patient had a decreased probing pocket depth and a negative Bleeding on probing index compared to initial clinical data, and the results were stable after 1 year. After 5 years of follow-up visits, there appeared to be rebound of the bone level radiographically. The author concluded by the limits of this case report, conventional non-surgical periodontal therapy with the adjunctive use of an 810-nm diode laser may be a feasible alternative approach for the management of peri-implantitis.

10. **Aykol G et al (2011);** In this study the author evaluated the effect of low-level laser therapy (LLLT) as an adjunct to non-surgical periodontal therapy of

patients with moderate to advanced chronic periodontitis. The LLLT group received GaAlAs diode laser therapy with a wavelength of 808 nm; Energy density of 4 J/cm2 was applied to the gingival surface after periodontal treatment on the first, second, and seventh days. In order to investigate the effect of smoking on treatment, each of the LLLT and control groups was divided into two groups as smoking and non-smoking patients. Gingival crevicular fluid samples were collected from all patients and clinical parameters include sulcus bleeding index (SBI), clinical attachment level, and probing depth (PD) levels were recorded on baseline, the first, third, and sixth months after treatment. From GCF samples matrixmetalloproteinase-1, tissue inhibitor matrix metalloproteinase-1, transforming growth factor-b1, and basic-fibroblast growth factor levels were measured. In this study the primary outcome was gingival bleeding and inflammation. At all-time points, the LLLT group showed significantly more improvement in sulcus bleeding index (SBI), clinical attachment level, and probing depth (PD) levels compared to the control group (P <0.001). In smokers and non-smoker's also there were clinically significant improvement in the laser-applied smokers' PD and SBI levels compared to smokers to whom a laser was not applied, between the baseline and all time points (P <0.001) (SBI score: control group 1.12, LLLT group 1.49; PD: control group 1.21 mm, LLLT group 1.46 mm, between baseline and 6months). Basic-fibroblast growth factor levels significantly decreased in both groups in the first month after the treatment, then increased in the third and sixth months (P <0.005). To conclude that LLLT as an adjunctive therapy to non-surgical periodontal treatment improves periodontal healing.

RESULT AND OBSERVATIONS

This in-vitro study was conducted on 120 sterile titanium discs contaminated with *P. gingivalis.* The antimicrobial effect of different treatment protocols on biofilm formation of *P. gingivalis* is presented in Table 1. It shows the count of colony forming unit/ml in all the groups (i.e., Control group, Indocyanine group (ICG), Photothermal therapy (PTT) and Photodynamic therapy (PDT). It was observed that most contaminated group was control group (ster-C) in which no treatment protocol was applied. In the present study it was observed that there was a significant reduction in P. gingivalis colony count in ICG, PTT and PDT group when compared with the control group. (Table 1). The maximum reduction in bacterial colony count was noted with PDT while minimum reduction was noted in ICG group. Therefore, it is described that Photodynamic therapy (PDT) showed maximum reduction in *P. gingivalis* bacterial colony count when compared to other treatment protocol.

Table 1: COLONY FORMING UNIT

COLONY FORMING UNIT – CFU / ml			
CONTROL	**INDOCYANINE GREEN DYE**	**LASER (BIOLASE)**	**PHOTODYNAMIC THERAPY**
60×10^4	52×10^4	28×10^4	24×10^4
64×10^4	48×10^4	36×10^4	20×10^4
60×10^4	56×10^4	40×10^4	20×10^4
56×10^4	52×10^4	28×10^4	16×10^4
64×10^4	52×10^4	36×10^4	20×10^4
68×10^4	48×10^4	40×10^4	20×10^4
60×10^4	52×10^4	32×10^4	24×10^4

60×10^4	52×10^4	32×10^4	24×10^4
64×10^4	48×10^4	36×10^4	20×10^4
60×10^4	56×10^4	28×10^4	20×10^4
56×10^4	52×10^4	32×10^4	16×10^4
64×10^4	52×10^4	36×10^4	24×10^4
68×10^4	48×10^4	40×10^4	20×10^4
60×10^4	52×10^4	32×10^4	24×10^4
68×10^4	52×10^4	40×10^4	16×10^4
64×10^4	48×10^4	36×10^4	20×10^4
60×10^4	56×10^4	28×10^4	20×10^4
56×10^4	52×10^4	32×10^4	16×10^4
64×10^4	52×10^4	36×10^4	24×10^4
68×10^4	48×10^4	28×10^4	20×10^4
68×10^4	52×10^4	32×10^4	20×10^4
60×10^4	48×10^4	32×10^4	20×10^4
60×10^4	52×10^4	36×10^4	16×10^4
64×10^4	52×10^4	28×10^4	24×10^4
60×10^4	48×10^4	32×10^4	20×10^4
56×10^4	56×10^4	36×10^4	24×10^4
64×10^4	52×10^4	40×10^4	16×10^4
68×10^4	52×10^4	32×10^4	20×10^4
60×10^4	48×10^4	40×10^4	20×10^4
60×10^4	48×10^4	40×10^4	20×10^4

Table 2: Descriptives Analysis of CFU/ml of all groups.

DYE: Indocyanine green dye; PTT: Photothermal therapy; PDT: Photodynamic therapy; Ster-C: Sterile control group

GROUPS	n	Mean ± SD	95% Confidence Interval	
			Lower Bound	Upper Bound
STER -C	30	$62.3 \pm 3.2 \times 10^4$	60.3×10^4	63.4×10^4
DYE	30	$51 \pm 2.6 \times 10^4$	50.9×10^4	52.1×10^4
PTT	30	$34.3 \pm 4.2 \times 10^4$	32×10^4	35.7×10^4
PDT	30	$20.7 \pm 2.7 \times 10^4$	19.4×10^4	21×10^4

* Shows a statistically significant result.

The descriptive analysis of all groups was done to estimate the mean and standard deviation. (Table 2). All test groups show significant differences compared to control group. Among test groups; Photodynamic therapy (PDT) protocol showed statistically significant reduction in microbial load compared to PTT and Dye groups, demonstrating that PDT was superior in reducing microbial load compared to other test groups. In comparison with mean and standard deviation of all test groups; the mean and standard deviation in PDT group is $20.7 \pm 2.7 \times 10^4$; stating that PDT is superior in reducing microbial load. With PTT and ICG(PS) the mean and standard deviation is $34.3 \pm 4.2 \times 10^4$ and $51 \pm 2.6 \times 10^4$ respectively; stating that PTT protocol is better in reducing the microbial load compared to ICG(PS);(Table 2).

Table 3: ANOVA

CFU	Sum of Squares	df	Mean Square	F	p-value
Between Groups	3.07E+12	3	1.02E+12	847.65	<0.001
Within Groups	1.4E+11	116	1.21E+09		

Analysis was done to see the difference between inter and intra-group comparison. ANOVA was carried out to determine the difference between and within groups (Table 3). Between the groups the mean difference was 3.07+12 and within groups the mean difference was 1.4+11. For individual result, inter group comparisons (i.e., multiple comparison) were carried out to determine the mean differences of control and test groups. It shows that there is a statistically significant *P value* of <0.001 between groups. (Table 3).

Table 4: Intergroup comparisons of control and test groups.

Multiple Comparisons

Dependent Variable: CFU

LSD

STATUS	STATUS	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
STER-C	DYE	10.3	8975.06	<0.001	91557.1	127109.6
STER-C	PTT	28	8975.06	<0.001	262223.8	297776.2
STER-C	PDT	41.6	8975.06	<0.001	400890.4	436442.9
DYE	PTT	17.6	8975.06	<0.001	152890.4	188442.9
DYE	PDT	30.3	8975.06	<0.001	291557.1	327109.6
PTT	PDT	13.6	8975.06	<0.001	120890.4	156442.9

Result of the Post hoc test between experimental groups according to *P-value*. The level of significance was set to 0.5. DYE: Indocyanine green dye; PTT: Photothermal therapy;

PDT: Photodynamic therapy; Ster-C: Sterile control group

*The mean difference is significant at the 0.05 level.

The inter group comparisons between Control and other test groups (Dye, PDT, PTT) showed mean difference of Dye – 10.3; PDT - 41.6; PTT – 28 compared to control groups. In comparison with Photosensitizer (ICG) and Photothermal therapy (PTT), PTT showed significant mean difference of 17.6; stating that PTT is better in reducing microbial load compared to ICG. Then comparing photosensitizer dye and photodynamic therapy, PDT showed significant mean difference of 30.3; stating that PDT is superior in reducing the microbial load compared to Photosensitizer dye (ICG).

In comparison with Photodynamic therapy and photothermal therapy; PDT showed significant mean difference of 13.6 stating that PDT is superior in reducing the microbial load compared to PTT. Therefore, the intergroup comparison shows that photodynamic therapy is superior in reducing the bacterial load when compared to other treatment groups and has statistically significant *P value* (<0.001) (Table 4)

MATERIALS AND METHODS

MATERIALS AND METHODS

The present in vitro study was undertaken to access and compare the effect of Indocyanine green, photothermal therapy and anti-microbial photodynamic therapy on titanium adherent biofilm of *Porphyromonas gingivalis.*

This study was conducted in the Department of Periodontics KAHER's KLE Vishwanath Katti Institute of Dental sciences and KLE's Dr. Prabhakar Kore Basic Science Research Centre (BSRC), KLE Academy of Higher Education and Research, Belagavi, Karnataka.

SOURCE OF DATA:

Titanium Discs: Commercially available pre-sterilised Grade 2 machined Titanium discs was procured from Indident™ (Indident Medical Devices, New Delhi).

Bacterial strain: *Porphyromonas gingivalis* (PG) – ATCC 33277 was obtained from depository of KLE's Dr. Prabhakar Kore Basic Science Research Centre (BSRC), KLE Academy of Higher Education and Research, Belagavi, Karnataka.

Indocyanine Green Dye: Commercially available Indocyanine green was procured from Aurogreen®, Aurolab, Madurai, Tamil Nadu.

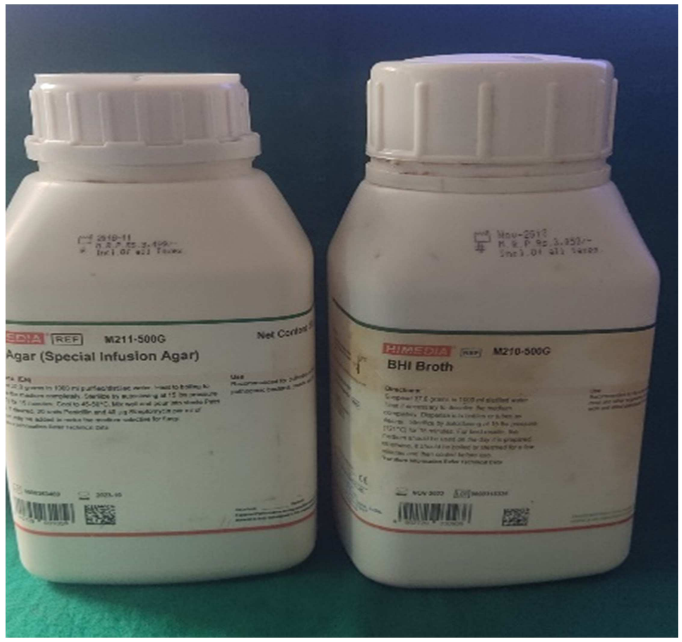
Laser: GaAlAs Diode Laser (BIOLASE, the Diode Laser Therapy System) is available in Department of Periodontics KAHER's KLE Vishwanath Katti Institute of Dental sciences, Belagavi, Karnataka.

ARMAMENTARIUM USED IN STUDY

MICROTITRE PIPETTE

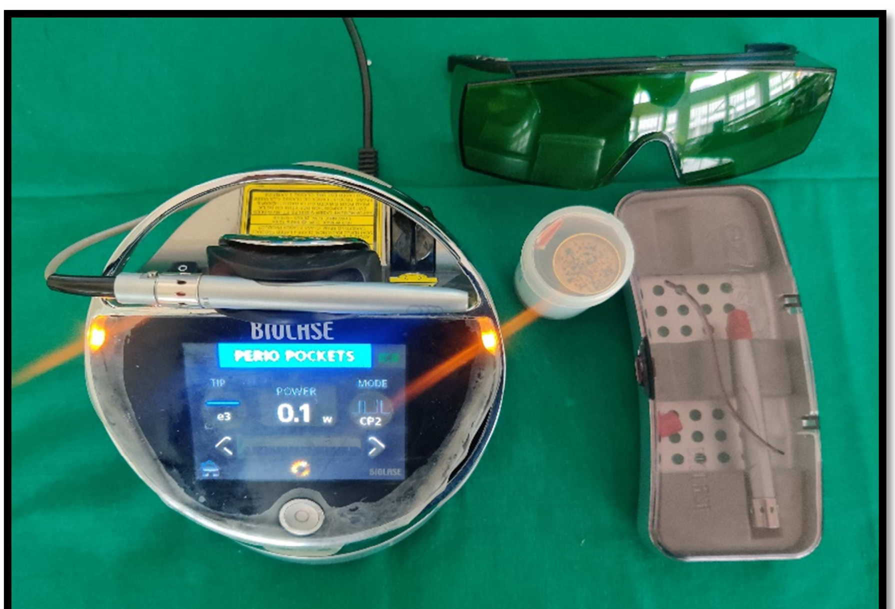
TITANIUM DISC

REAGENT USED



BHI BROTH & BHI AGAR

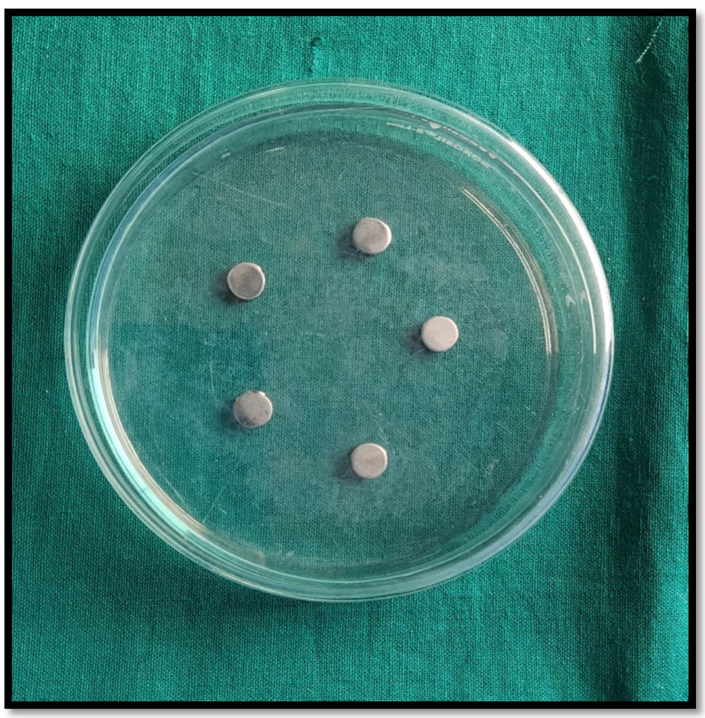
LASER



DIODE LASER (BIOLASE)

PHOTOSENSITIZER DYE

INDOCYANINE GREEN DYE



PRE-STERILIZED TITANIUM DISC

METHOD OF COLLECTION DATA:

Methodology

Inoculum preparation: Inoculum preparation was carried out in BHI broth. Standard bacterial colonies of the same morphological type of *Porphyromonas gingivalis* were taken from a cultured agar plate. Further, each colony was picked with the help of sterile loop, and the grown bacteria were transferred to a falcon tube having 5 mL of BHI broth. This broth culture was further incubated at 37°C for 8–14 hours. This bacterial strain then transferred to the disc which was placed in petri plates.

Bacterial strain and colonization: The attachment and maturation of the bacteria on the disc surface eventually produce an extracellular substance (matrix) and lead to a complex organization of cells called the biofilm. These bacterial biofilms were studied without washing the disc with 1× PBS leaving the biofilm structure intact after incubation period of 48 hours.

Bacterial formation was accessed by using Phloxin B stain.

Titanium disc preparation: 120 Pre sterilized titanium discs of diameter 8 mm and thickness of 2 mm was obtained from Indident™ (Indident Medical Devices) and randomly allocated to four different group. Each group contains 30 discs.

- Group 1 - Control group
- Group 2 – Photosensitizer (Test group 1)
- Group 3 – Photothermal therapy (Test group 2)
- Group 4 – Photodynamic therapy (Test group 3)

Blood agar was prepared in Petri-plates and the titanium discs are placed in the prepared petri -plates and the strains *Porphyromonas gingivalis* (PG) – ATCC 33277 which was obtained from depository BSRC was inoculated on thirty titanium discs. The discs are kept in an anerobic chamber for 48 hours and 72 hours.

Bacterial colonization or biofilm formation was taken places in all discs. This biofilm formation on titanium discs was assessed by Phloxin – B stain.

All these inoculated discs in Petri plates were randomly allocated to four different groups that includes

- Group 1: Control group (no treatment);
- Group 2: Photosensitizer (Indocyanine green dye ICG);
- Group 3: Photothermal Therapy (Diode Laser);
- Group 4: Photodynamic Therapy (ICG + Diode Laser)

Laser protocol

Titanium discs was irradiated with GaAlAs Diode Laser (BIOLASE, the Diode Laser Therapy System) with 400μm fiber optic handpiece at a wavelength of 940 nm operated at power – 1 W, with a pulse length of 200μm and pulse interval of 200μm in noncontact mode for 30-60s.

Indocyanine green

A solution of Indocyanine green was prepared by dissolving it in 5 mL of sterile water to prepare an initial 5 mg/ mL ICG stock solution. This stock solution was further diluted in saline solution at ratio of 1:5 to achieve final ICG concentration of 5mg/mL before implementation.

Photodynamic therapy procedure

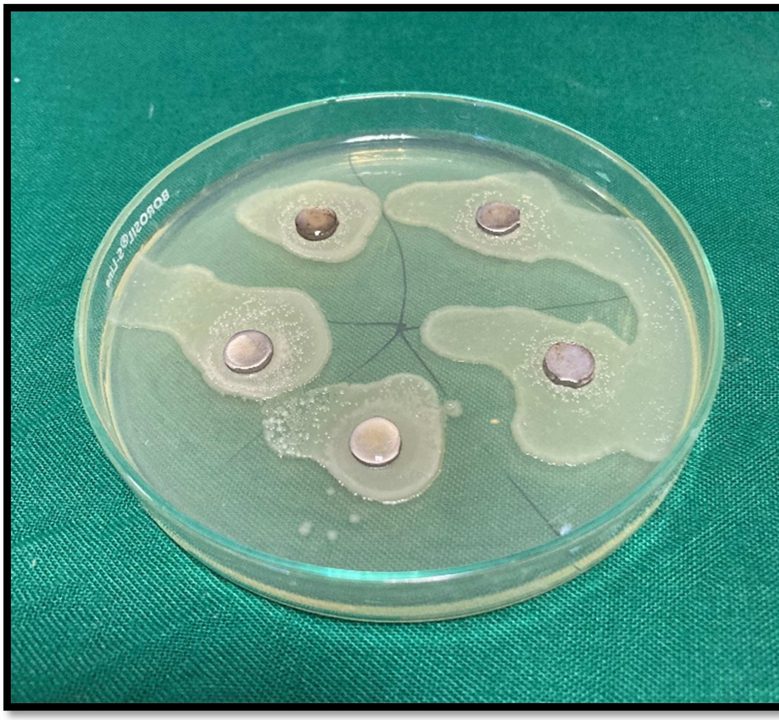
Application - Titanium discs which are randomly allocated with solution for 2 minutes.

Soaking phase – The solution with active ingredient attaches the bacterial cell membrane and dyes them; sensitize the bacteria.

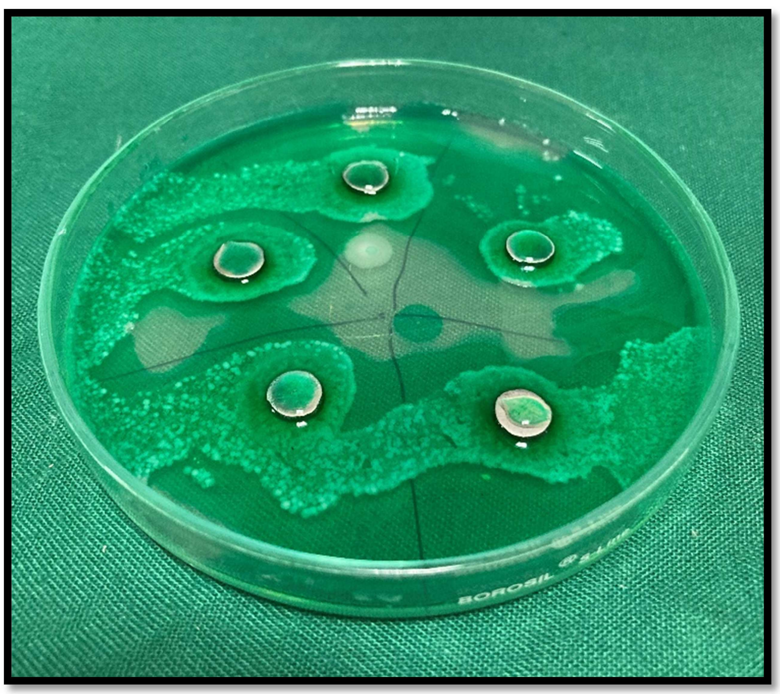
Rinsing phase – Rinsing off excessive active ingredients. Green dye bacteria remain on titanium discs.

Activation - It is activated by laser light energy for 30-60 s.

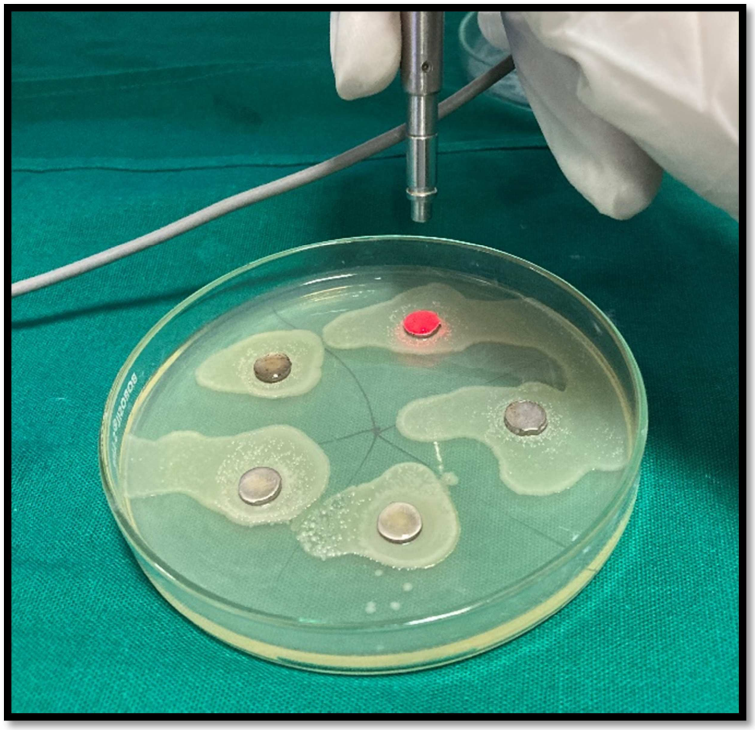
INOCULATED TITANIUM DISC WITH *P.GINGIVALIS* STRAIN



INOCULATED TITANIUM DISC WITH DYE



INOCULATED TITANIUM DISC WITH LASER



INOCULATED TITANIUM DISC WITH PHOTODYNAMIC THERAPY

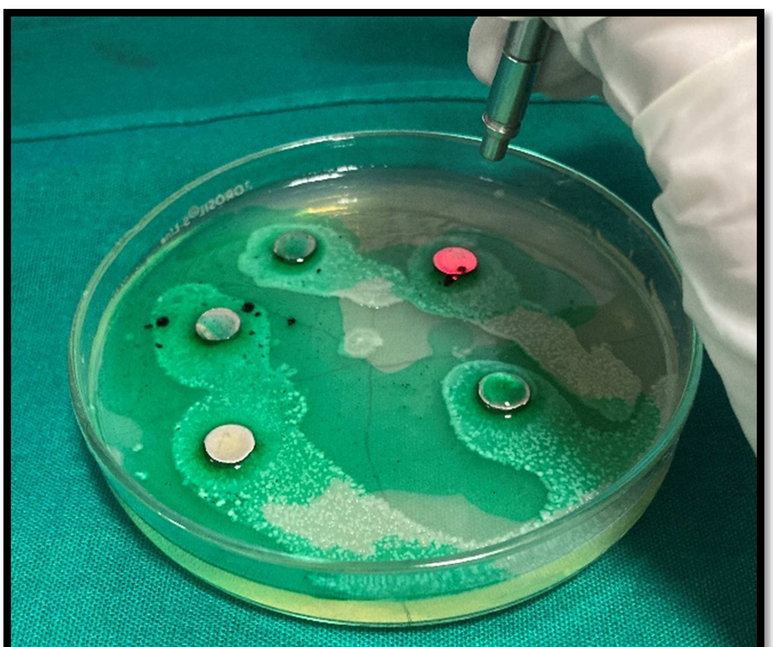
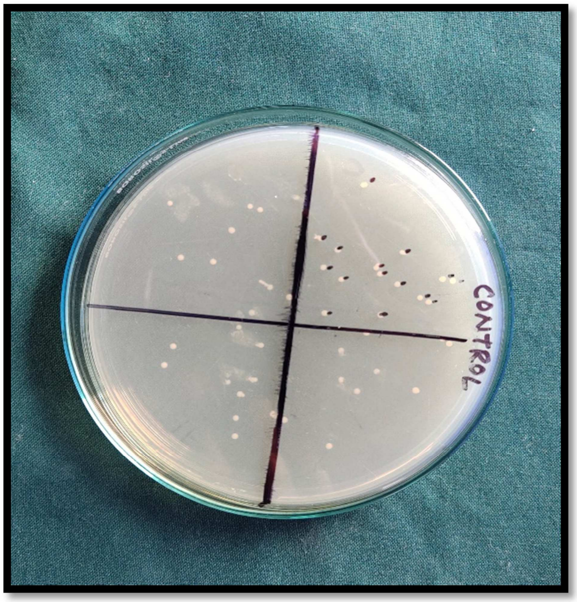
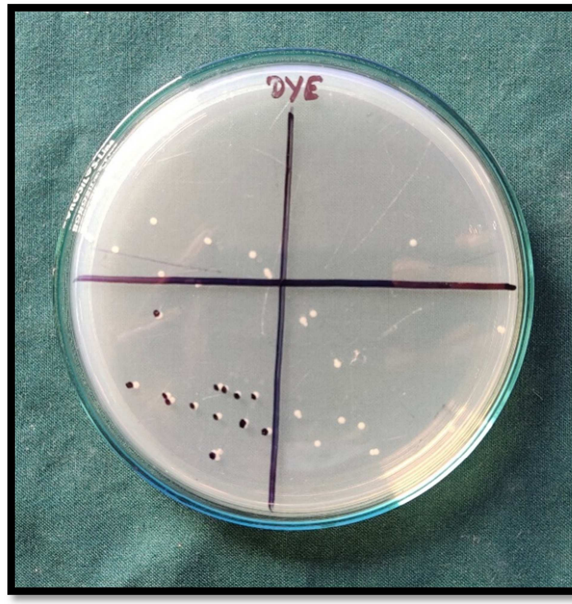


PLATE COUNT METHOD

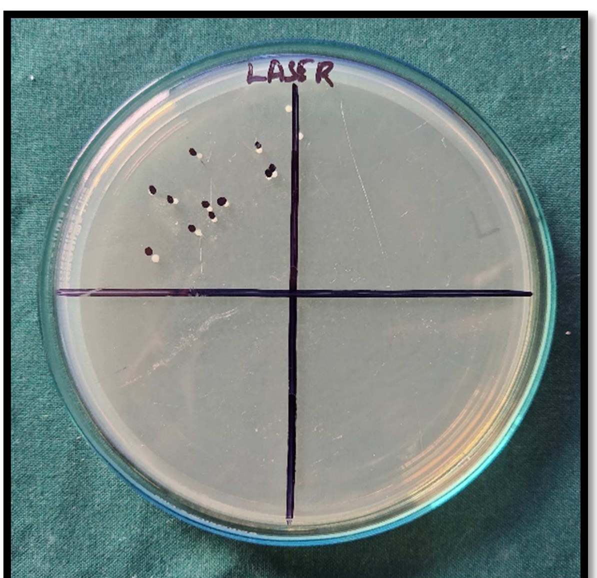
CONTROL



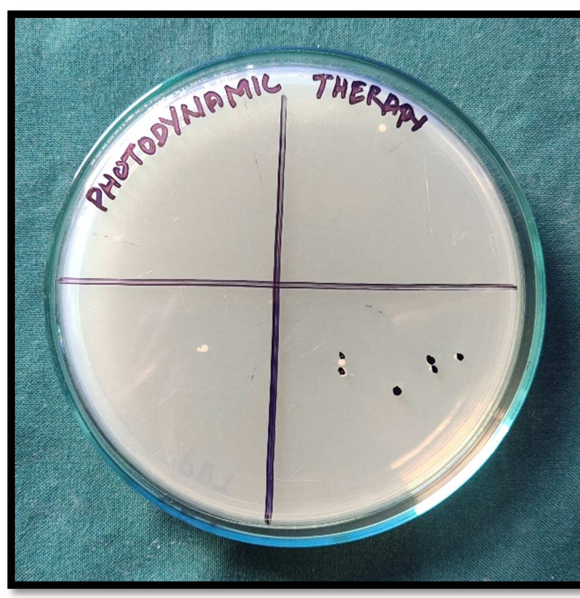
INDOCYANINE GREEN DYE



PHOTOTHERMAL



PHOTODYNAMIC THERAPY



STATISTICAL ANALYSIS

The data were entered in Microsoft Excel and analyzed statistically using the SPSS software, version 21; SPSS Inc., (Chicago, IL, USA). The normality of the data was assessed prior to analysis using the Shapiro-Wilk's test/Kolmogorov-Smirnov test. Data were found to be normally distributed. Thus, parametric test was chosen. Descriptive analysis was calculated.

Inter group comparisons was done by One-way ANOVA. Pairwise Comparison of four groups was also carried out using LSD post hoc test. All statistical tests were performed at a significance level of 5% ($p<0.05$).

DISCUSSION

Several studies and investigations have been conducted to find an alternative method for conventional treatment for peri-implantitis, which consists of mechanical debridement of the involved implant surfaces. Among all microbes engaged in peri-implant diseases, the major periodontopathogens is *P. gingivalis* for developing periodontitis and peri-implant disease.

The current study was carried out to compare and examine the outcome of Indocyanine green (ICG), Photothermal therapy (PTT), and Photodynamic therapy (PDT) against *P.gingivalis*. The results showed significant reductions in colony counts of *P.gingivalis* on the titanium surfaces in all studied groups. The maximum reduction was observed with Photodynamic therapy and the lowest rate was seen in the ICG group. It was also found that the PTT also showed a statistically significant reduction in *P.gingivalis* colony count compared to the ICG group. (Table1).

It was observed that all test groups (PDT, PTT, ICG) showed a statistically significant reduction in *P.gingivalis* colony count when compared with the control group (Table 2). The maximum reduction in colony count was noted with PDT while the minimum reduction was noted in the ICG group. These observed outcomes were in line with the study done by Alagl et al [24] states that PDT showed a significant reduction in microbial load compared to photothermal therapy.

Evidence shows that aPDT decreases tumor necrosis factor alpha and interleukin-1B[9]. This is in accordance with the present study stating that the efficacy of PDT with ICG and diode laser showed a statistically significant reduction of *P.gingivalis* bacterial colony count. A clinical study has shown that in the application of PDT there is a reduction in the level of cytokines in gingival crevicular fluid[25]. It also resolves inflammation and enhances tissue healing[26]. Evidence shows that all photosensitizer molecules (i.e. cationic, anionic, and neutral) play a significant role in the killing of Gram-positive bacteria and Gram-negative bacteria[27]. Gram-negative bacteria have an internal cytoplasmic membrane along with an outer membrane, which resists the penetration of photosensitizer. ICG is an anionic photosensitizer, which is water soluble and relatively non-toxic[28]. Its photothermal effect is greater than its photochemical effect[29]. It can efficiently reduce the bacteria from deep periodontal pockets due to its photothermal effect. Its absorbance peak should be at 800 -940 nm[30]. Thus, the Diode laser used in this current study is suitable for ICG i.e., PDT[17](Table 2).

Bohem et al.[31] demonstrated that *A. actinomycetemcomitans* had the maximum absorbance peak in presence of a 10 µm concentration of ICG for 5 min. In his study; a diode laser used with 810 nm with 0.1W and 0.5 W power at 80 and 400 W/cm2 energy density for a time period of 5s caused a reliable reduction in *A. actinomycetemcomitans* in a culture medium. Only laser therapy did not show a reduction in the bacterial count. Because the power of the laser (0.1W) used was of lower Watt and the time period of application was of shorter duration(5s). In this current study, the diode laser was used with 940nm at power (150 mW) for a time period of the 30s. Our study showed a significant reduction of *P. gingivalis* colony count on titanium discs (Table 1); stating that the use of higher power watts and longer duration showed a statistically significant reduction in the bacterial count with both laser and Photodynamic therapy. (P value < 0.001) (Table 2)

Pourhajibagher et al.[32] demonstrated that ICG at 62.5 µg/mL concentrations for 5 min caused a 23.2% reduction in *P. gingivalis* count. Diode laser irradiation with 810 nm wavelength and 62.5 J/cm2 energy density for 2 min caused a reduction in *P. gingivalis* count by 37%. In this current study, we used ICG at 1000 µg/mL concentration for 5 mins caused a marked reduction in *P. gingivalis* count and its Mean ± SD value is 20.7 ± 19.4. Diode laser irradiation with 940nm wavelength and 6 J/cm^2 energy density for 60 sec caused a reduction in *P. gingivalis* count with a Mean±SD value of 34.3 ± 4.3 and shows statistically significant results in both laser and PDT groups *(P value* < 0.001). (Table 2).

Topaloglu et al.[33] have shown the positive efficacy of PDT with ICG for cutting down *Staphylococcus aureus* (95%) and *Pseudomonas aeruginosa* (99%). Their results were in accordance with our study but the organism investigated was *P. gingivalis*; they had evaluated the resistant microbial suspension of different bacterial species. It seems that the gram-negative bacteria hold out longer to PDT than gram-positive bacteria because of the presence of the outermost membrane in gram-negative bacteria, which hampers the uptake of Photosensitizer dye. However, because of the anionic charge of ICG and the cationic charge of Toluidine blue-O(TBO), and Methylene blue (MB); it easily binds to the membrane of gram-negative bacteria and interacts with lipopolysaccharides. Thus, the Photosensitizer (ICG) used in our present study is suitable for reduction in the bacterial count of *P.gingivalis* which is one of the pathogens causing periodontitis and peri-implantitis. Diode lasers have deep penetration properties and the use of photosensitizer along with diode lasers has effectively reduced the microbial count of gram-negative bacteria. In this study, ICG-based PS used is a newly introduced photosensitizer in the dental field.

Saffarpour et al.[34;] Moslemi et al[35] and Mattiello et al[36]; have reported that PDT with diode laser with 810nm and 300mW power and 2.38 W/cm2 power density caused a statistically significant reduction in *A.actinomycetemcomitans* count on implant surfaces. These findings were in check with the present study stating that aPDT showed a reliable reduction in *P.gingivalis* colony count on implant surfaces ($P<0.001$) within the group and between the groups. (Table 3)

Birang et al[37,] in the year 2017 conducted a randomized clinical trial on peri-implantitis patients, both groups in his study i.e., Control (LT) and Test group (PDT) had shown statistically significant clinical parameters advancement (i.e., bleeding on probing, pocket depth (PPD) and modified plaque index). The number of *Aggregatibacter actinomycetemcomitans* ($P = 0.022$), *Tannerella forsythia* ($P = 0.038$), and *Porphyromonas gingivalis* ($P = 0.05$) in the experimental group showed a significant reduction in the bacterial count. These findings were in accordance with the present study and showed a statistically significant reduction of *P.gingivalis* colony count using Photodynamic therapy ($P<0.001$). (Table 4)

Giannelli et al[38,] did a study on various lasers to estimate the efficacy of the anti-microbial property of photodynamic therapy on the titanium-coated biofilm of *P.gingivalis.* The laser included in the study are Er:YAG (with 2,940-nm wavelength, a power density of 75.4 w/cm^{2}, and pulse energy of 100 mJ for 1 minute), Nd: YAG (with 1064 nm wavelength and maximum power density of 75.4 W/cm^2 for 1 minute), and diode laser with 810nm (in continuous mode with 1W power and power density of 175.4 W/cm^2 for 1 minute in photoablation mode.

It showed that a Diode laser in conjunction with Photosensitizer (methylene blue) showed a significant reduction in *P.gingivalis* on the surface of titanium discs compared with other lasers and confirmed the superiority of a PDT. These findings were in accordance with the present study which shows that PDT has significant results ($P<0.001$) over lasers. (Table 4). The advantage of using PS for the treatment of periodontitis or peri-implantitis is these materials can penetrate the implant and root surface porosities that are not accessible by mechanical debridement.

Thus, the bactericidal act of Photosensitizer (ICG) is mainly due to its photothermal effect. However, the photodynamic therapy of this material gained less attention since the oxygen pressure is low. The photothermal mechanism is effective in conjunction with PS(ICG) for reducing the microbial load on implant surfaces. As per the knowledge at present no studies have been done in evaluating the ICG – PDT on peri-implantitis.

In the present in-vitro study, it states that there was a significant depletion in *P.gingivalis* colony count on titanium disc PDT states that ICG with photodynamic therapy (ICG-PDT) showed statistically significant results in reducing antimicrobial-resistant strains causing periodontitis and peri-implantitis[39]. Collectively, all this evidence indicates that ICG-PDT provides a clinical benefit over conventional treatment. The main advantages of using ICG-PDT are a non-invasive surgical procedure that can penetrate deep tissue and can reduce the bacterial fill in the affected sites and has no side effects; it is suitable for medically compromised patients who are not indicated for surgical procedures[40,41]. It can act as an alternative advancement for reducing microbes on the implant surfaces and it could be used as an adjunct with conventional treatment.

LIMITATIONS

The present study mainly focused on the bactericidal efficacy of laser and PDT on *P.gingivalis* biofilm coated on titanium discs. Thus, to generalize the result of this study, more studies should be conducted on the effect of treatment modalities on the biofilm of other periodontal pathogens causing periodontitis and peri-implantitis.

Further studies with different photosensitizers and different laser parameters are required to determine the most efficient combination for aPDT.

None of the techniques in this study was capable of the complete elimination of *P.gingivalis* bacteria on the implant surface. There was a failure in the complete elimination of *P. gingivalis* on implant surfaces, stating that these methods are inadequate for decontamination of the implant surface, so it is still used in association with conventional treatment to eliminate the *P. gingivalis* biofilm.

The biocompatibility, thermal changes, and alterations that are seen in implant surfaces were not evaluated, so further studies on cell culture and animal models are needed for this purpose.

Finally, the result of this study (in-vitro) may not be generalized to in vivo conditions. Environmental factors such as variable plaque accumulation, salivation, immune system, limited accessibility, etc., cannot be established in in-vitro studies, so the result of this study could be established by in-vivo studies with large sample sizes.

SUMMARY AND CONCLUSION

The main focus of this study was to evaluate and compare the efficacy of photothermal therapy, indocyanine green, and antimicrobial photodynamic therapy against a periodontal pathogen namely *Porphyromonas gingivalis* that is adherent to titanium disc. The present study supports the hypothesis that there would be a significant effect of indocyanine green, photothermal therapy, and photodynamic therapy on the titanium adherent biofilm of *Porphyromonas gingivalis.* The findings of the current study conclude that Photothermal and photodynamic therapy can reduce the growth of *Porphyromonas gingivalis* on titanium discs.

Within the limitation of this present study, Photodynamic therapy had superior efficacy in reducing the *P.gingivalis* biofilm than the other methods investigated in the study. Simultaneously, photothermal therapy was also found to be a suitable method for disinfecting and reducing the colony count of *P.gingivalis* biofilm on titanium discs in comparison with photosensitizer alone. Therefore, PDT is an effective alternative treatment method for scaling the microbes on dental implant surfaces without damaging the surface topography and also could be used as a suitable additive step to conventional treatment. The result of this research requires to be further corroboration with long-term prospective in-vivo clinical trials.

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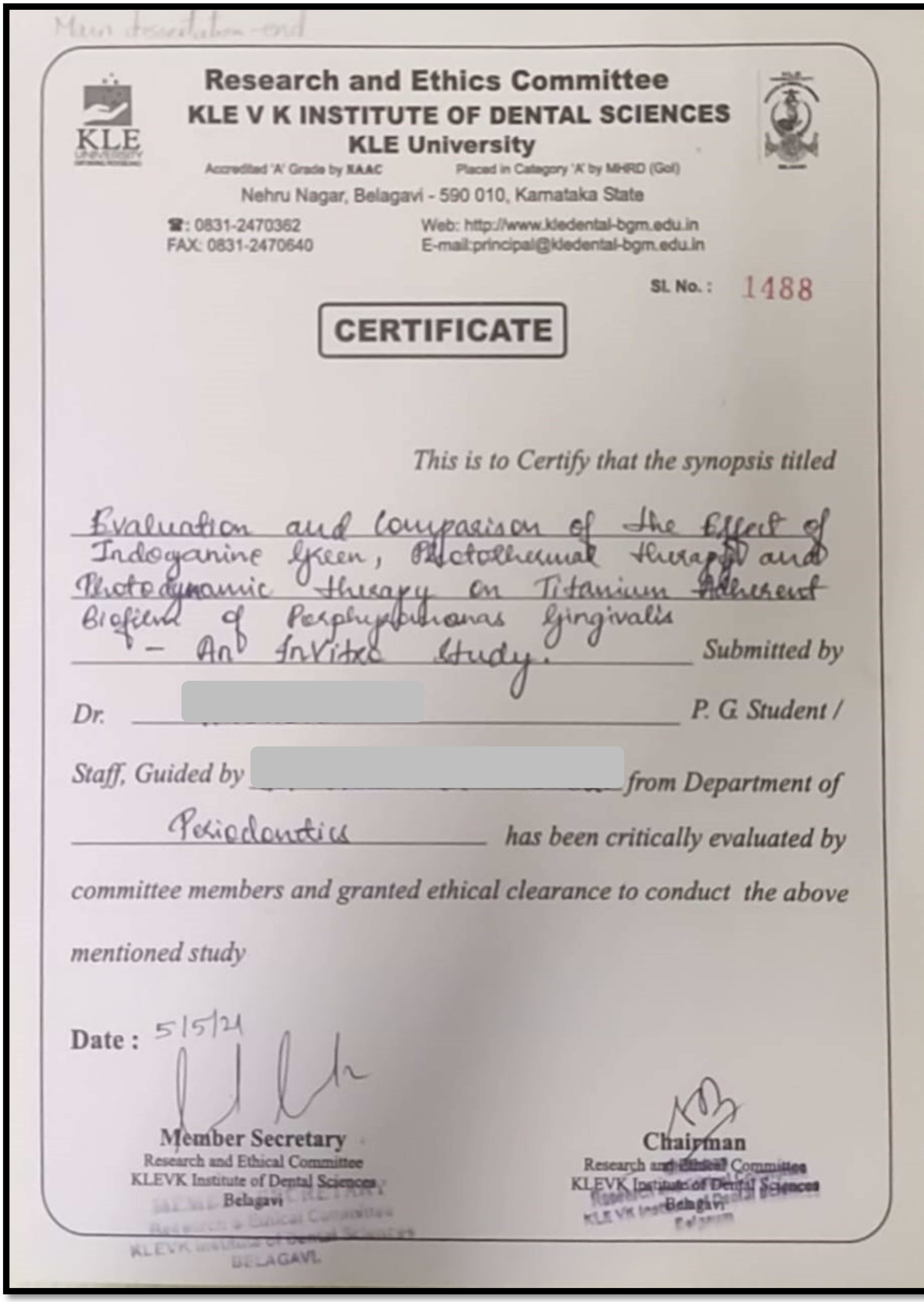
ANNEXURE I: ETHICAL CLEARANCE



Main dissertation - end

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
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.: 1488

CERTIFICATE

This is to Certify that the synopsis titled Evaluation and Comparison of the Effect of Indocyanine Green, Photothermal therapy and Photodynamic therapy on Titanium Adherent Biofilm of Porphyromonas Gingivalis - An In Vitro Study. *Submitted by Dr.* [redacted] *P. G Student / Staff, Guided by* [redacted] *from Department of* Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE II: COLONY FORMING UNIT - RESULT

KAHER's Dr. Prabhakar Kore Basic Science Research Center [BSRC]
KLE Academy of Higher Education and Research [KAHER]

Report

TITLE OF THE STUDY: Evaluation and Comparison of the effect of Indocyanine Green, Photothermal therapy and Photodynamic therapy on titanium adherent biofilm of *Porphyromonas gingivalis* - An In-Vitro Study

NAME OF THE STUDENT:

NAME OF THE GUIDE:

NAME OF THE CO-GUIDE:

STRAINS TESTED: *Porphyromonas gingivalis.*

OBJECTIVES:

To access and compare the effect of the photosensitizer, photothermal therapy and photodynamic therapy on titanium coated biofilm of *Porphyromonas gingivalis.*

RESULT:

Colony forming units is counted after performing test group procedure.

EXPERIMENTAL METHODOLOGY:

Pre sterilized Titanium discs of diameter 8 mm and thickness of 2 mm and *Porphyromonas gingivalis* strains is procured. *Porphyromonas gingivalis* will be inoculated on titanium discs. Bacterial colonization or Biofilm formation is taken place within 48 hours. After 48 hours incubated Titanium discs is placed in broth agar plate.

Discs with *Porphyomonas gingivalis* biofilm will be exposed to 1 mg/mL ICG at 37 °C in the dark for 5 min and then rinsed with sterile PBS for 30 s. Titanium disc will be irradiated with diode laser alone (940nm). Photosensitizer dye (ICG) adheres to titanium disc and is activated by diode laser.

After 48 hours, colony forming unit is measured by plate count method.

RESULT:

COLONY FORMING UNIT – CFU /ml

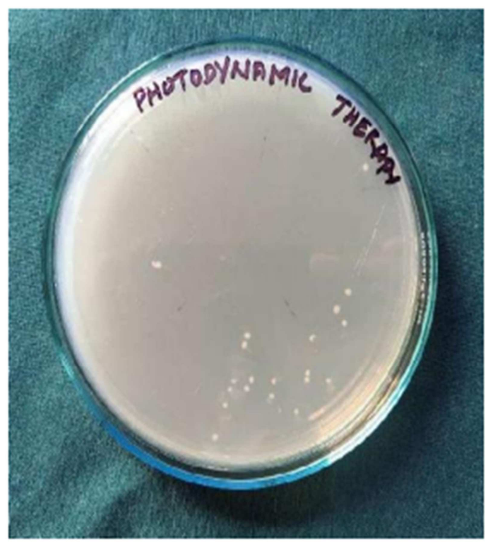
COLONY FORMING UNIT – CFU / ml			
CONTROL	**INDOCYANINE GREEN DYE**	**LASER (BIOLASE)**	**PHOTODYNAMIC THERAPY**
60×10^4	52×10^4	28×10^4	24×10^4
64×10^4	48×10^4	36×10^4	20×10^4
60×10^4	56×10^4	40×10^4	20×10^4
56×10^4	52×10^4	28×10^4	16×10^4
64×10^4	52×10^4	36×10^4	20×10^4
68×10^4	48×10^4	40×10^4	20×10^4
60×10^4	52×10^4	32×10^4	24×10^4
60×10^4	52×10^4	32×10^4	24×10^4
64×10^4	48×10^4	36×10^4	20×10^4
60×10^4	56×10^4	28×10^4	20×10^4
56×10^4	52×10^4	32×10^4	16×10^4
64×10^4	52×10^4	36×10^4	24×10^4
68×10^4	48×10^4	40×10^4	20×10^4
60×10^4	52×10^4	32×10^4	24×10^4
68×10^4	52×10^4	40×10^4	16×10^4
64×10^4	48×10^4	36×10^4	20×10^4
60×10^4	56×10^4	28×10^4	20×10^4
56×10^4	52×10^4	32×10^4	16×10^4
64×10^4	52×10^4	36×10^4	24×10^4
68×10^4	48×10^4	28×10^4	20×10^4

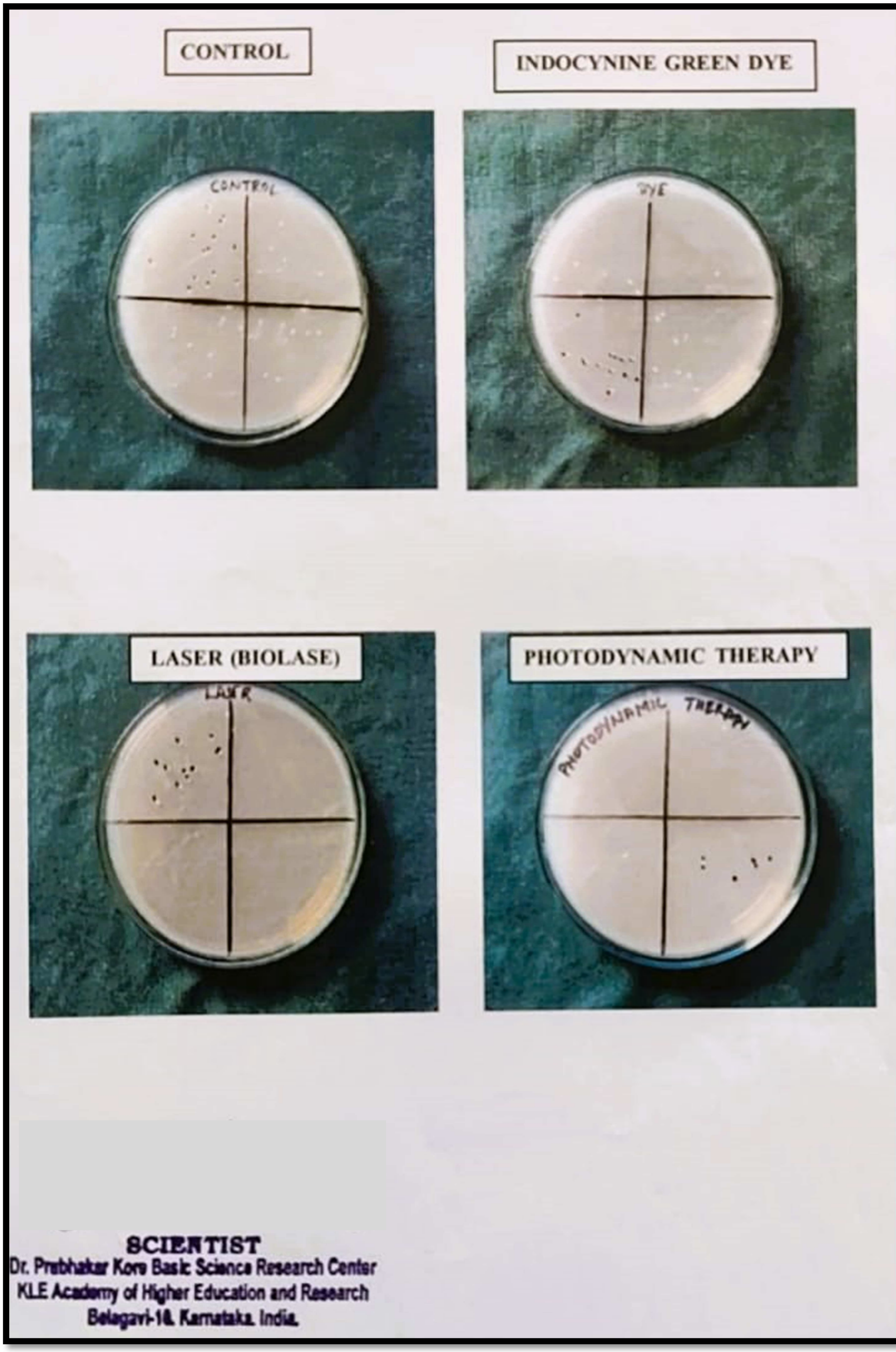
CONTROL

INDOCYNINE GREEN DYE

LASER (BIOLASE)

PHOTODYNAMIC THERAPY




CONTROL
INDOCYNINE GREEN DYE
CONTROL
DYE
LASER (BIOLASE)
PHOTODYNAMIC THERAPY
LASER
PHOTODYNAMIC THERAPY
SCIENTIST
Dr. Prabhakar Kore Basic Science Research Center
KLE Academy of Higher Education and Research
Belagavi-10, Karnataka, India.

ANNEXURE III: 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 (2nd Cycle) — Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640

Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Date : 3.11.2022 — Serial No. : 110

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : POSTGRADUATE
Batch & Year : 2020 - 2023
Department : ORAL PATHOLOGY

The soft copy of Research Work / Manuscript by .... entitled "EVALUATION AND COMPARISON OF THE EFFECT OF INDOCYANINE GREEN PHOTOTHERMAL THERAPY AND PHOTODYNAMIC THERAPY ON TITANIUM ADHERENT BIOFILM OF PORPHYROMONAS GINGINALIS – AN INVITRO STUDY" 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 ....2..........%, which is **within** / ~~**not within**~~ the acceptable limits of 10% as per the UGC guidelines.

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

ANNEXURE IV: BIOSTATISTICIAN CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
: 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 the Biostatistics aspect of the Dissertation / Research work of **Post Graduate Student**, under the guidance of M.D.S, **Professor, Department of Periodontics**, entitled **"Evaluation And Comparison of The Effect of Indocyanine Green, Photothermal Therapy and Photodynamic Therapy on Titanium Adherent Biofilm Of *Porphyromonas Gingivalis* - An In Vitro Study"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 15·06·22

Name & Signature of Biostatistician

DEPARTMENT OF EPIDEMIOLOGY & BIOSTATISTICS KAHER'S BELAGAVI