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Effect of Low-Level Laser of 650nm on Viability of Porphyromonas Gingivalis with and with Out Photosensitizers: An Invitro Study

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Abstract:- Porphyromonas gingivalis is a major pathogen associated with initiation and progression of periodontitis.

- ***Aim and Objective***

The aim of the study is to compare effect of low-level laser of wavelength 650nm on viability of porphyromonas gingivalis with and without photosensitisers.

- ***Methods***

Thirty samples of bacterial suspensions (200µl) were prepared and divided into six groups .Group 1- laser group (low level laser with wavelength of 650nm, and irradiation time of 30 s), Group 2- Methylene blue + laser group (after pre-irradiation time of 10 min, laser was irradiated), and Group 3- Toluidine blue O + laser group (after pre irradiation time of 10 min, LED was irradiated), Group 4 -Methylene blue Group 5 - Toluidine blue O (TBO) and Group 6- Control group (no treatment), Then, 20 µL of each sample was cultured in blood agar plates and incubated for 72 hours in microaerophilic atmosphere for colony counting.

- ***Result***

The concentration of P. gingivalis was significantly reduced after using the Methylene blue + laser and the Toluidine blue O + laser group. (P values < 0.005)

- ***Conclusion***

Within the constraints of this investigation, it can be deduced that photodynamic inactivation applying a laser and photosensitizers like Methylene blue and toluidine blue was more efficient than photosensitizers and laser irradiation alone in eliminating P. *gingivalis*.

Keywords:- *Porphyromonas Gingivalis , Low Level Laser , Methylene Blue , Toluidine Blue O.*

I. INTRODUCTION

Periodontal disease is an inflammatory process of the tissues surrounding the teeth. Bacterial plaque is the main cause of periodontitis. [1] Porphyromonas gingivalis , a black pigmented microorganism, is a major pathogen associated with initiation and progression of periodontitis. [2]

Antimicrobial photodynamic therapy (aPDT) has been introduced as an alternative approach for antibacterial therapy. It is a combination of 2 nontoxic ingredients, a photosensitizer and light, which destruct the cell by photodamage, and finally leads to cell death. [3,4] A photosensitizer (a photoactivable material) attaches to the target cells and gets stimulated by a proper wavelength of light.

P. *gingivalis* , which has endogenous photosensitizer molecules such as porphyrins that in the presence of light generate reactive oxygen species leading to bacterial killing.

On the other hand, some studies using an exogenous photosensitizer implicated the capacity of aPDT for killing P. *gingivalis*. [5] These photo sensitizers once stimulated by Low level laser (650 nm) releases free radicals of oxygen which are cytotoxic. [6].

This study was carried out to evaluate and compare the influence of two different photosensitizing agents with Low level laser on the viability of P. *gingivalis* .

II. MATERIALS AND METHODS

The samples used for the study was ATCC- 33277 strains of Porphyromonas gingivalis obtained from the laboratory where the study was conducted. (Laboratory: Central research laboratory, Maratha mandal dental college, Belgavi, Karnataka). The laser used was Baistra Portable F3WW PAD Dental Low Level Laser model no 1600100100 (110v-220v)

P. *gingivalis* strains was suspended in thioglycolate broth and bacterial density was visually adjusted to a turbidity of 0.5 McFarland standard reagents. 200 µl of bacterial suspension was transferred in to microtiter plates. The wells of the microtiter plates were diluted with 1000 µl distilled water. The wells were divided in to 6 groups.

- *Group I: Laser alone* (Wells contained 200 µL bacterial suspension and 200 µL broth. Then laser was irradiated to the wells.)

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- *Group 2: Methylene blue with Laser* (Wells contained 200 µL bacterial suspension and 200 µL MB. After pre-irradiation time of 10 min, Laser was irradiated to bacterial suspensions)
- *Group 3: Toludine blue with Laser* (Wells contained 200 µL bacterial suspension and 200 µL TBO. After pre-irradiation time of 10 min, Laser was irradiated to bacterial suspensions)
- *Group 4: Methylene blue alone* (Wells contained 200 µL bacterial suspension and 200 µL MB)
- *Group 5: Toludine blue alone* (Wells contained 200 µL bacterial suspension and 200 µL TBO.)
- *Group 6: Negative control* (Wells contained 200 µL bacterial suspension and 200 µL broth. Control wells were not treated by either light sources or photosensitizers.)

Wells corresponding to respective groups were filled with photosensitisers (200µl) and incubated at room temperature for 10 minutes followed by laser irradiation for 30 seconds. Photosensitizers were then removed and culture well plates were added with 1000 µl of thioglycolate broth. After another 10 minutes of incubation at room temperature 20µl of the inoculum from the broth is sub-cultured into the culture media plate containing Blood agar and incubated at 37°C for 72 hours. CFU per millilitre was quantified.

III. RESULT

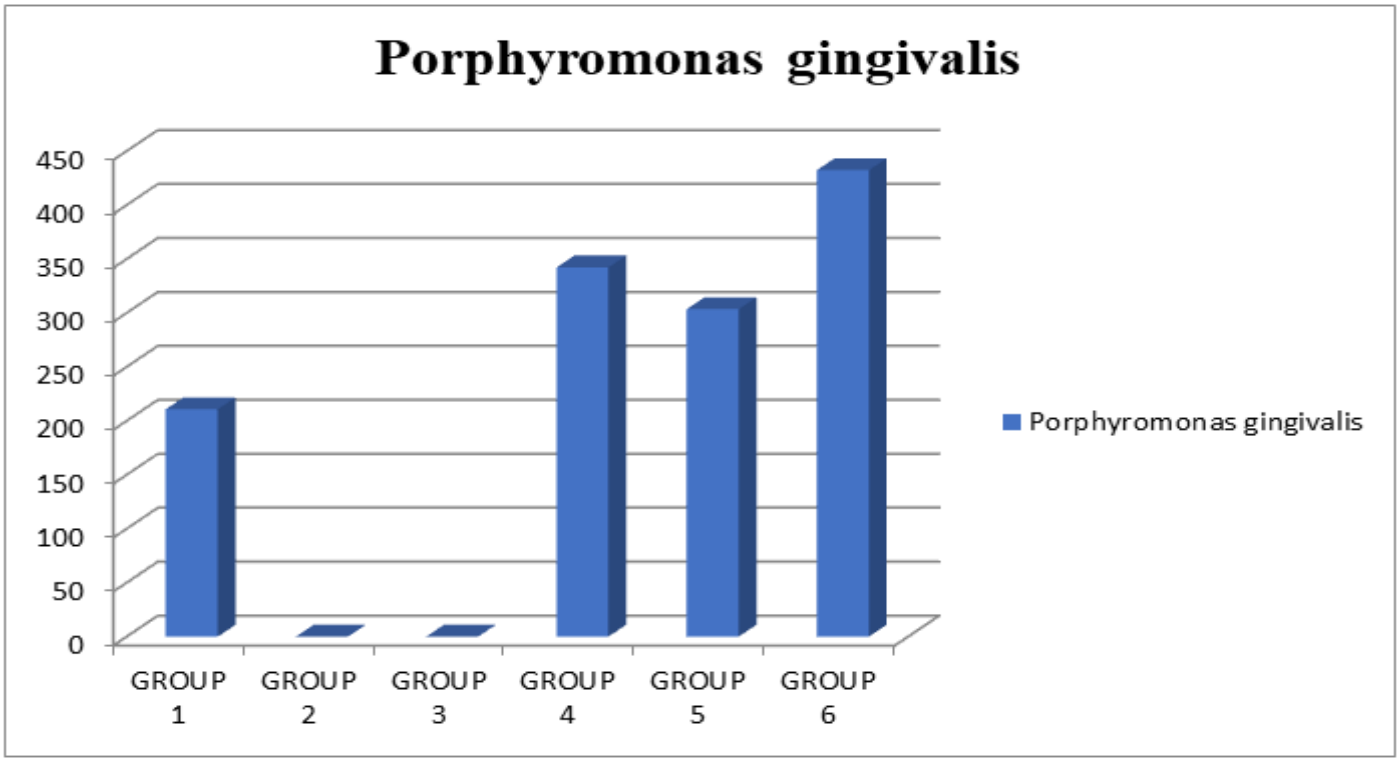
The result of the study indicates that Group 2 and 3 (Laser + Methylene and Laser + Toluidine blue O) had no growth of P. *gingivalis* when compared to Group 1,4,5 (Laser alone, Methylene blue alone and Toluidine blue O alone) which showed significant reduction in the CFU/mL of P. *gingivalis* .

Table 1 demonstrates Mean±SD values of logarithm of CFU/mL in each treatment group. Laser-based photodynamic therapy significantly reduced the number of CFU/mL in comparison to the control group.

Statistical analysis demonstrated that administration of Laser + MB and Laser + TBO resulted in no growth of the microorganism. However, Laser only, methylene blue and toluidine blue only groups demonstrated reduced bacterial count in comparison with control group. **(Graph 1)**

Table 1 Shows Six Groups in Porphyromonas Gingivalis with Mean and Standard Deviation. P Value in Comparison with Control Group

	Porphyromonas gingivalis		
	n	**Mean ± SD**	**P value**
GROUP 1	5	210.60 ± 26.950	.000*
GROUP 2	5	.00 ± .000	.000*
GROUP 3	5	.00 ± .000	.000*
GROUP 4	5	342.00 ± 35.249	.000*
GROUP 5	5	303.40 ± 35.211	.000*
GROUP 6	5	432.20 ± 12.853	control



Graph 1: Graphical representation of bacterial count in comparison with each group

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IV. DISCUSSION

Bacterial infection plays an important role in the development of periodontal disease; thus, the main purpose of periodontal treatment is the elimination of supra and subgingival bacterial colonization, which initiate inflammation in the periodontal tissues. [7]

Porphyromonas gingivalis, one of the red complex bacteria is one of the major pathogens that are responsible for periodontitis progression [8] is a Gram-negative, anaerobic, rod-shaped bacteria forming black colonies on blood agar. It was detected in 85.75% subgingival plaque of chronic periodontitis patients [9] and is known to produce a wide array of virulence factors that could cause tissue destruction on their own or act through other mediators to induce inflammation. P. gingivalis can efficiently modify the host immune response and create an environment favourable to its own and other pathogens' continued persistence. [10]

Primary goal of periodontal therapy is Eliminating all bacterial deposits on the tooth surface. From varying efficacy of debridement to antibiotic resistance, development of alternate approach to eradicate bacteria from periodontal pocket was crucial.

Photodynamic inactivation of microorganisms is based on the concept that a dye, known as a photosensitizer (PS), localized preferentially in the bacteria and not in the surrounding tissues or cells, and subsequently activated by low doses of visible light of an appropriate wavelength to generate free radicals or singlet oxygen that are toxic to target microorganisms. [5]

The result of this investigation showed that Laser + Methylene blue and Laser + Toluidine blue O and resulted in statistically significant decrease in the concentration of P. *gingivalis* . This is in accordance with **Jahangirnejad M et al** , where colonies of P. *gingivalis* reduced significantly and even disappeared after 15 seconds photosensitizing agents with laser irradiation where in control (No laser irradiation) , colony count remain without change .

The findings of this study indicated that laser irradiation with photosensitizers such as Methylene blue and Toluidine blue O reduced the microorganisms in comparison to the control, laser alone and photosensitizers alone. Laser with photosensitizers showed no growth of P. *gingivalis* while there was statically significant reduction in the groups with laser irradiation alone, photosensitizers alone and control group.

You Chan et al & Chern-Hsiung Lai et al investigated the bactericidal effects of photodynamic therapy and concluded that laser with Methylene blue could eliminate up to 40% of bacteria on average than the control group of Methylene blue alone .

Neda Moslemi et al stated that laser-based photodynamic therapy had a great reduction in colony count of *P. gingivalis* in comparison with Radachlorin® or laser irradiation alone.

V. CONCLUSION

Photodynamic therapy with different photosensitisers has tremendous potential in inhibiting and reducing the growth of P. *gingivalis* and other bacteria associated with periodontal disease. Further clinical researches on the efficacy of low-level laser and various concentrations of photosensitizing agents are required prior to conclude any clinical benefit of photodynamic therapy.

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