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RESEARCH ARTICLE

EFFECT OF LOW LEVEL LASER OF 650nm ON VIALBILITY OF PREVOTELLA INTERMEDIAWITH AND WITHOUT PHOTOSENSITISERS: AN IN VITRO STUDY

Dr. M.M Dayakar[1], Dr. Mohammed Nihal Abbas Majeed[2] and Dr. Apoorva Gurulingappa[3]

1. Professor and Head of the Department, Department of Periodontology, KVG Dental College and Hospital, India.
2. Post Graduate Student, Department of Periodontology, KVG Dental College and Hospital, India.
3. Post Graduate Student, Department of Periodontology, KVG Dental College and Hospital, India.

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Abstract

Background: Prevotella intermedia is commonly associated with various forms of periodontal disease. Conventional treatment involves a combination of mechanical therapy and systemic antibiotics administration, which comes with its own set of challenges and disadvantages. An alternative treatment modality is low-level laser therapy with and without photosensitizers.

Purpose: This study aimed to evaluate the effectiveness of low-level laser therapy with and without the use of photosensitizers, specifically Methylene blue and Toluidine blue, on the viability of Prevotella intermedia.

Methods: The study utilized in vitro experiments to assess the impact of low-level laser therapy alone and in combination with photosensitizers on the viability of Prevotella intermedia.

Results: The findings indicate that Methylene blue, when combined with low-level laser therapy, exhibits bactericidal effects similar to or greater than low-level laser therapy alone on Prevotella intermedia. Additionally, the use of Toluidine blue as a photosensitizer in combination with low-level laser therapy also led to a reduction in the viability of Prevotella intermedia, although it was not as effective as Methylene blue.

Conclusion: Low-level laser therapy, particularly when combined with Methylene blue as a photosensitizer, shows promise as a potential treatment modality for controlling Prevotella intermedia in periodontal diseases.

Introduction:-

Prevotella intermediasensulato, characterized as an obligate anaerobic, black-pigmented, gram-negative rod, is commonly linked to various periodontal conditions, including adult periodontitis, acute necrotizing ulcerative gingivitis, and pregnancy gingivitis.[1,2]

Corresponding Author:- Dr. Mohammed Nihal Abbas Majeed
Address:- Post Graduate Student, Department of Periodontology, KVG Dental College and Hospital, India.

Despite the high morbidity associated with aggressive periodontitis, there is no established protocol for the efficient control of this disease, and the outcome of treatment is uncertain. The conventional treatment includes the combination of mechanical therapy and the administration of systemic antibiotics.[3] However, a widespread issue with antibiotic administration is the lack of patient compliance. Furthermore, ensuring a sustained drug concentration in the periodontal pocket for a specific duration presents another obstacle concerning the use of antimicrobial agents.[4]Additionally, it is essential to acknowledge the potential adverse effects associated with antibiotic usage, including allergic reactions, gastrointestinal disturbances, and the development of bacterial resistance.[5]

Hence, various alternative treatment approaches have been suggested. One such modality is low-level laser therapy, a safe method that utilizes a photosensitive molecule binding to cells and becoming activated by specific light wavelengths. During activation, the photosensitizer's energy transitions to a heightened state. Thereafter, cytotoxic singlet oxygen and free radicals are generated, which are damaging to important components of the cells and microorganisms, such as plasma membrane and DNA.[6–9]

The purpose of this study was to evaluate if low-level laser exposure affects the growth of Prevotella intermedia with and without photosensitizers.

Materials and Methods:-

Institutional Ethics Committee of KVG Dental College and Hospital reviewed and discussed the application to conduct the study entitled "Effects of low level laser of 650nm on viability of PrevotellaIntcrmcdiawith and without photoscnsitizers: An in vitro study" and approved it.

In this in-vitro investigation, an ATCC culture of Prevotella intermedia (ATCC No. 25611D-5) was employed, obtained from the Central Research Laboratory at Maratha Mandal Dental College, Belgaum, Karnataka. The utilized laser device was the Baistra Portable F3WW PAD Dental Low Level Laser, model number 1600100100 (110v - 220v). The Prevotella intermedia bacteria were suspended in a Thioglycolate broth, and the bacterial density was adjusted to a turbidity of 0.5 McFarland standard using visual assessment. A volume of 200 µl of the bacterial suspension was introduced into microtiter plates. These plate wells were subsequently diluted with 1000 µl of distilled water, and the wells were categorized into six distinct groups.

Group 1: Laser-Only Group; Group 2: Methylene blue + Laser Group; Group 3: Toluidine blue + Laser Group; Group 4: Methylene blue Group; Group 5: Toluidine blue Group; Group 6: Negative Control Group.

After photosensitizers (200 µl) were introduced into the respective wells and left at room temperature for a duration of 10 minutes before exposure to laser light for a period of 30 seconds. After the photosensitizers were removed, 1000 µl of Thioglycolate broth was supplemented to the wells. Following another 10-minute incubation at room temperature, 20 µl of the inoculum from the broth was sub-cultured onto a culture media plate containing blood agar. This culture was then incubated for 72 hours at 37°C. Colony Forming Units (CFU) per milliliter were subsequently determined.

Results:-

The highest Colony Forming Units (CFU) were observed in Group VI followed by Group V while there was no growth observed for Group I and Group II. In addition, Group III and Group IV also had very minimum growth. This difference was found to be statistically significant ($P = 0.001$). A post hoc Tukey's test was conducted and it revealed that there was NO statistically significant difference between Group I vs Group II ($P = 0.99$), Group I vs Group III ($P = 0.99$), Group I vs Group IV ($P = 0.99$), Group II vs Group III ($P = 0.99$), Group II vs Group IV ($P = 0.99$), and Group III vs Group IV ($P = 0.99$). In addition, a statistically significant difference in mean CFU was found between Group I vs Group V ($P = 0.001$), Group I vs Group VI ($P = 0.001$), Group II vs Group V ($P = 0.001$), Group II vs Group VI ($P = 0.001$), Group III vs Group V ($P = 0.001$), Group III vs Group VI ($P = 0.001$), Group IV vs Group V ($P = 0.001$), Group IV vs Group VI ($P = 0.001$), and between Group V vs Group VI ($P = 0.001$) respectively. In other words, Group I, Group II, Group III, and Group IV reduced the growth of Prevotella intermedia. Though Group V was effective when compared to Group VI, Group V had more CFU when compared to Group I to Group IV respectively.

Figure 1:- Mean Colony Forming Unit for Prevotella Intermedia according to different groups(Original).

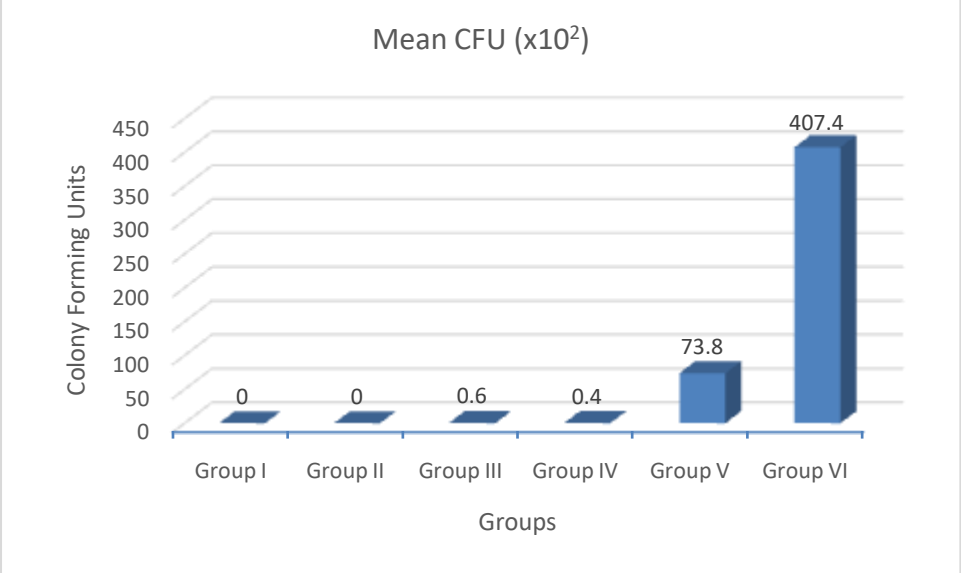

Table 1:- Comparison of mean Colony Forming Units ($x10^2$) against Prevotella Intermedia between the groups(Original).

	Mean	**SD**	**F**	**P value**
Group I	0	0		
Group II	0	0		
Group III	0.6	0.5		
Group IV	0.4	0.5	2075.11	P = 0.001**
Group V	73.8	8.6		
Group VI	407.4	17.55		

SD-standard deviation
**Statistically significant at P < 0.01 using One Way ANOVA

Table 2:- Multiple comparisons between the groups(Original).

		MD	P value
Group I	Group II	0	P = 0.99
	Group III	-0.6	P = 0.99
	Group IV	-0.4	P = 0.99
	Group V	-73.8	P = 0.001**
	Group VI	-407.4	P = 0.001**
Group II	Group III	-0.6	P = 0.99
	Group VI	-0.4	P = 0.99
	Group V	-73.8	P = 0.001**
	Group VI	-407.4	P = 0.001**
Group III	Group IV	0.2	P = 0.99
	Group V	-73.2	P = 0.001**
	Group VI	-406.8	P = 0.001**
Group IV	Group V	-73.4	P = 0.001**
	Group VI	-407	P = 0.001**
Group V	Group VI	-333.6	P = 0.001**

MD-Mean Difference
**Statistically significant using Post hoc Tukeys test

Discussion:-

The development of periodontitis has been strongly linked to specific bacterial species and their combinations, including AggregatibacterActinomycetemcomitans, PorphyromonasGingivalis, Prevotella Intermedia,

TreponemaDenticola, and Tannerella Forsythia.[10–12] The prevalence of periodontopathogenic microorganisms in periodontal pockets was illustrated as follows: Porphyromonasgingivalis 24%, Aggregatibacteractinomycetemcomitans 23%, Prevotella intermedia 20%, Tannerella forsythia 22%, Treponemadenticola 11%.[13]

In its function as a disclosing agent, Methylene blue (MB) operates as a phenothiazine dye with the ability to stain biofilms. Apart from its staining properties, MB demonstrates antimicrobial effects that are intensified by light absorption. Its antibacterial effects are mediated through DNA damage, yet it remains non-toxic to humans.[14]Prior research has indicated that Toluidine Blue O (TBO) serves as an efficient photosensitizer against a range of bacteria, including those typically present in the oral cavity.[15,16]

The present study aimed to investigate the effects of low-level laser of 650nm on the viability of Prevotella intermedia with and without photosensitizers. The experimental setup included six distinct groups: Laser-Only Group, Methylene blue + Laser Group, Toluidine blue + Laser Group, Methylene blue Group, Toluidine blue Group, and Negative Control Group. The Laser-Only Group served as a control to evaluate the effects of the laser alone on the viability of Prevotella intermedia. In the Methylene blue + Laser Group and the Toluidine blue + Laser Group, the Prevotella intermedia bacterial suspension was combined with either Methylene blue or Toluidine blue as photosensitizers, followed by exposure to the 650nm low-level laser for 30 seconds. The researchers also included a Methylene blue Group and a Toluidine blue Group, where the bacterial suspension was combined with either Methylene blue or Toluidine blue without exposure to the laser. The use of photosensitizers in combination with low-level laser therapy has been shown to have bactericidal effects on periopathogenic bacteria. The findings of the current study revealed that the application of low-level laser therapy by itself had a notable impact on the viability of Prevotella intermedia. This observation aligns with prior research demonstrating the species-specific response of bacteria to low-level laser therapy. Moreover, employing Methylene blue as a photosensitizer alongside low-level laser therapy yielded a comparable decrease in the viability of Prevotella intermedia. This discovery implies that when Methylene blue is combined with low-level laser therapy, it exhibits bactericidal effects that are comparable to or even greater than those of low-level laser therapy alone on Prevotella intermedia. Furthermore, employing Toluidine blue as a photosensitizer alongside low-level laser therapy also resulted in a decrease in the viability of Prevotella intermedia, albeit to a lesser extent compared to Methylene blue. This contrasts with previous studies suggesting that Toluidine blue typically demonstrates greater bactericidal activity than Methylene blue against most bacteria under both dark and light conditions.[17] Our results suggest that Toluidine blue does not augment the bactericidal effects of low-level laser therapy on Prevotella intermedia. Unlike Methylene blue, Toluidine blue did not exhibit a synergistic effect when combined with low-level laser therapy, indicating that the choice of photosensitizer plays a critical role in enhancing the bactericidal effects of the 650nm low-level laser on Prevotella intermedia. When interpreting the microbiological effects obtained through antimicrobial photodynamic therapy (aPDT), one must consider the potential outcomes resulting from the administration of the photosensitizer itself. In our study, Methylene blue alone demonstrated superior bactericidal effects compared to Toluidine blue alone. Other studies have shown PDT significantly inhibited the growth of P. intermedia which is in agreement with our study.[18,19]Multiple assessments indicate that in vitro, PDT showcases antibacterial properties against several periodontal pathogens,[20] and it also reduces the biological activity related to LPS production by pathogenic bacteria.[21] However, It's crucial to note the limited availability of data from controlled clinical investigations that directly compare the use of aPDT with non-surgical periodontal therapy, as well as studies comparing aPDT, scaling and root planing (SRP), or the photosensitizer alone (without light activation). Before making any definitive conclusions regarding the potential clinical and microbiological benefits of incorporating aPDT into non-surgical therapy, further research with a substantial sample size is necessary.

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