International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

# Effects of Low Level Laser of 650nm on Vialbility of Aggregatibacter Actinomycetemcomitans with and without Photosensitisers: An Invitro Study

**Dr. M M Dayakar[1], Dr. Apoorva. G[2], Dr. Devipriya B[3], Dr. Nihal Abbas[4]**

[1]Professor and Head of the Department, Department of Periodontology, KVG Dental College and Hospital

[2, 3, 4]Post Graduate Student, Department of Periodontology, KVG Dental College and Hospital

**Abstract:** ***Aggressive periodontitis (Grade C periodontitis) is significantly correlated with Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans). The aim of this investigation was to compare the effect of low level laser of 650nm on vialbility of aggregatibacter actinomycetemcomitans with and without photosensitisers. Thirty samples of bacterial suspensions (200µl) were prepared and divided into six groups: laser group (low level laser with wavelength of 6oonm, and irradiation time of 30 s), Methylene blue + laser group (after pre - irradiation time of 10 min, laser was irradiated), and Toluidine blue O + laser group (after preirradiation time of 10 min, LED was irradiated), Methylene blue group (0.1 %), Toluidine blue O (TBO) group (0.1 mg/ mL) and control group (no treatment), Then, 100 µL of each sample was cultured in blood agar plates and incubated for 72 h in microaerophilic atmosphere for colony counting. Application of Methylene blue + laser and Toluidine blue O + laser group resulted in a significant decrease in the concentration of A. actinomycetemcomitans (P values< 0.005) Within the limits of this study, it can be concluded that photodynamic inactivation using laser and Photosensitizers like Methylene blue and Toulidine blue was more effective than photosensitizers and laser irradiation alone in eradication of A. actinomycetemcomitans.***

**Keywords:** Methylene blue; Toluidine blue O; Low level laser; Aggregatibacter actinomycetemcomitan

## 1. Introduction

Aggregatibacter *actinomycetemcomitans* a highly non - motile gram - negative coccobacillus with a vast array of potential virulence factors and mechanisms is identified as an important factor for onset of aggressive periodontitis, which is responsible for the progressive destruction of supporting structures of the teeth and if not treated, can eventually lead to early edentulism. [1] [2]

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 administration of systemic antibiotics [3]. However, poor patient compliance is a common problem associated with administration of antibiotics. The difficulty to maintain a sufficient drug concentration in the periodontal pocket for a certain time is another problem related to the use of antimicrobial agents [4]. In addition, the potential adverse effects related to the use of antibiotics (such as allergic reactions, gastric upset, and emergence of bacterial resistance) must be noted [5]. Therefore, some alternative treatment modalities have been proposed.

Low level laser therapy is a safe treatment modality based on the use of a photosensitive molecule that adheres to the cells and gets activated by light of the appropriate wavelength. Throughout the activation procedure, the energy of photosensitizer shifts to a higher 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 Several photosensitizers and light sources have been applied for inactivation of A. actinomycetemcomitans. [6] [7] [8] [9]

The purpose of this study is that to evaluate if the low - level laser exposure has effect on growth of Aggregatibacter *actinomycetemcomitans* with and without photosensitisers.

## 2. Materials and Method

This invitro study was done using a ATCC culture of A. *actinimycetemcomitans (*ATCC cultures 43718) obtained from Central research laborartory, Maratha Mandal dental college, Belgaum, Karnataka. The laser used was Baistra Portable F3WW PAD Dental Low Level Laser model no 1600100100 (110v - 220v).

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

**Group 1**: laser alone - wells contained 200 µL bacterial suspension and 200 µL broth. Then laser was irradiated to the wells.

**Group 2**: Methylene blue + laser - - wells contained 200 µL bacterial suspension and 200 µL MBO. After pre - irradiation time of 10 min, Laser was irradiated to bacterial suspensions

**Group 3**: Toulidine blue + 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 - wells contained 200 µL bacterial suspension and 200 µL MBO

**Group 5**: Toulidine blue – 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.

Photosensitizers (200µl) were poured into the appropriate wells and allowed to remain at room temperature for 10 minutes before being exposed to laser light for 30 seconds.1000µl of thioglycolate broth should be added to the wells after the photosensitizers have been eliminated. After another 10 minutes of incubation at room temperature, 20 l of the inoculum from the broth is sub - cultured into a culture media plate containing Blood agar and incubated for 72 hours at 37 $^{0}$C. CFU in a millilitre was measured.

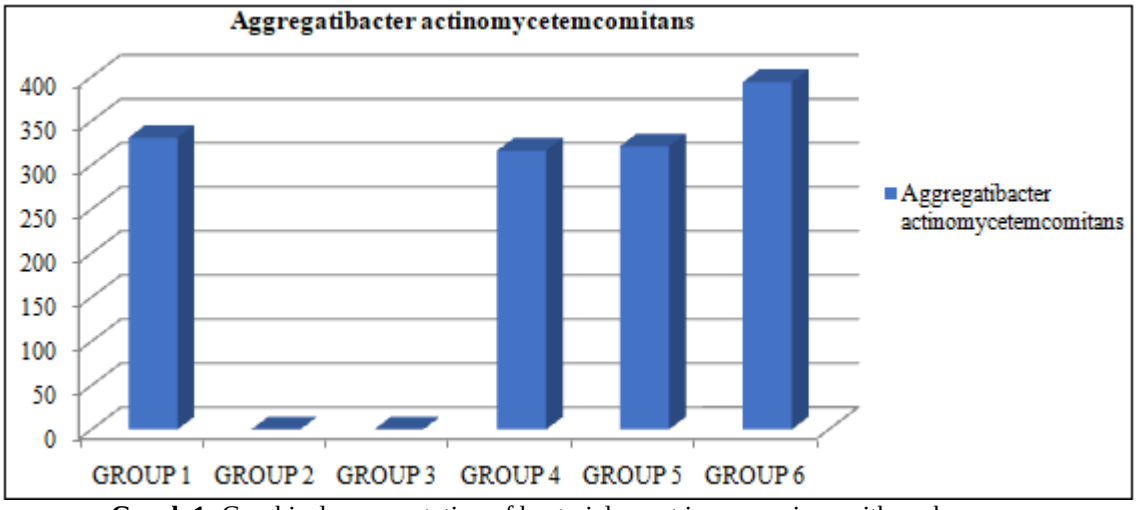
## 3. Result

Table 1 shows 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 + MBO and Laser + TBO resulted in no growth of the microorganism. However, Laser only, methylene blue and toulidine blue only groups demonstrated reduced bacterial count in comparison with control group. (Graph 1)

**Table 1:** Shows 6 groups in Aggregatibacter actinomycetemcomitans with mean and standard deviation. P value< 0.05 in comparison with control group

| Aggregatibacter *actinomycetemcomitans* | | | |
|---|---|---|---|
| GROUP | n | Mean + SD | P value |
| GROUP 1 | 5 | 331.00 + 28.8 | 0.018* |
| GROUP 2 | 5 | 0.00 + 0.00 | 0.00* |
| GROUP 3 | 5 | 0.00 + 0.00 | 0.00* |
| GROUP 4 | 5 | 316.40 + 51.3 | 0.003* |
| GROUP 5 | 5 | 321.40 + 31.6 | 0.005* |
| GROUP 6 | 5 | 394.20 + 17.7 | Control group |




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

## 4. Discussion

The standard treatment for aggressive periodontitis remains highly unspecific, depending mostly on the mechanical debridement of the affected root surfaces in conjunction with antimicrobial drugs. However, a small, although relevant proportion of sites and patients do not respond adequately to this therapy. The regular use of antimicrobial medications, however, has led to reports of bacterial strains evolving into resistant ones more recently. The traditional therapeutic approaches may be replaced with antimicrobial photodynamic therapy (aPDT). A photoactivable substance (the photosensitizer) attaches to the target cell in aPDT, where it is triggered by light of the right wavelength. Free radicals are created during the activation process, and they have a damaging effect on the cell. [10]

The results of this investigation demonstrated that laser + Methylene blue and laser + toulidine blue O resulted in a statistically significant decrease in the concentration of A. actinomycetemcomitans.

Many studies have investigated the effect of different combinations of lasers and photosensitizers on A. actinomycetemcomitans. In the presence or absence of Malachite green (MG), A. actinomycetemcomitans was subjected to a 30mW diode red laser. Two laser exposure times—t=3 min (energy dose=5.4 J/cm) and t=5 min (energy dose=9 J/cm) were employed in the presence of MG. Bacterial colonies were enumerated and transformed into colony forming units after the samples were diluted. Between the two energy doses used, significant differences were seen ($p<0.05$). Both the red laser and MG by alone were unable to eradicate microorganisms. These findings show that red laser and MG may photosensitize A. actinomycetemcomitans and that the dye is photodegraded after exposure to light. [11]. Rose Bengal (RB) photosensitizer dye in concentrations up to 0.1 micromol L did not show toxicity per se toward A.

actinomycetemcomitans cells and the reduction in the biofilm (about 45%) is significantly dependant on RB concentration and irradiation time when this dye was used as a ROS generator. [12]

The results of this study demonstrated that the reduction of the microorganism in comparison with the control growth with laser irradiation with photosensitizers like methylene blue and toulidine blue demonstrated no growth of Aggregatibacter actinomycetemcomitans and this is in accordance with the study done by Goulart Rde et al, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans were fully destroyed by methylene blue at concentrations >10 g/mL and red light - emitting diode irradiation for 10 s from a distance of 22 mm. [13] Chan and Lai demonstrated that irradiation of 665 - nm diode laser in the presence of methylene blue resulted in approximately 95 % killing of A. actinomycetemcomitans [14].

The potential effects resulting from the application of the photosensitizer itself should be taken into account when interpreting the microbiologic effects acquired with aPDT. It should also be noted that there is very little information from controlled clinical studies that compares the use of aPDT with non - surgical periodontal therapy to the use of aPDT, SRP, or photosensitizer alone (i. e., without light activation). Before any conclusive conclusions can be made regarding the potential clinical and microbiological benefits of aPDT used in conjunction with non - surgical therapy, additional studies with a significant sample size are required.

## 5. Conclusion

Within the constraints of this investigation, it can be said that PDI employing laser and photosensitizers (MBO and TBO) was more successful than Laser and photosensitizers alone in eliminating A. actinomycetemcomitans.

## References

[1] Newman MG, Takei H, Klokkevold PR, Carranza FA. Newman and Carranza's Clinical periodontology E - book. Elsevier Health Sciences; 2018 May 29.
[2] Raja M, Ummer F, Dhivakar CP. Aggregatibacter actinomycetemcomitans–a tooth killer?. Journal of clinical and diagnostic research: JCDR.2014 Aug; 8 (8): ZE13.
[3] Deas DE, Mealey BL. Response of chronic and aggressive periodontitis to treatment. Periodontology 2000.2010 Jun 1; 53: 154 - 66.
[4] Feres M, Haffajee AD, Allard K, Som S, Goodson JM, Socransky SS. Antibiotic resistance of subgingival species during and after antibiotic therapy. Journal of clinical periodontology.2002 Aug; 29 (8): 724 - 35.
[5] Slots J, Ting M. Systemic antibiotics in the treatment of periodontal disease. Periodontology 2000.2002 Jan; 28 (1): 106 - 76.
[6] Calin MA, Parasca SV. Light sources for photodynamic inactivation of bacteria. Lasers in medical science.2009 May; 24 (3): 453 - 60.
[7] Novaes AB, Schwartz - Filho HO, de Oliveira RR, Feres M, Sato S, Figueiredo LC. Antimicrobial photodynamic therapy in the non - surgical treatment of aggressive periodontitis: microbiological profile. Lasers in medical science.2012 Mar; 27 (2): 389 - 95.
[8] Maisch T. Anti - microbial photodynamic therapy: useful in the future?. Lasers in medical science.2007 Jun; 22 (2): 83 - 91.
[9] De Paula Eduardo C, de Freitas PM, Esteves - Oliveira M, Aranha AC, Ramalho KM, Simões A, Bello - Silva MS, Tunér J. Laser phototherapy in the treatment of periodontal disease. A review. Lasers in medical science.
[10] Raab O (1900) The effect of fluorescent agents on infusoria (in German). Z Biol 39: 524–526.
[11] Prates RA, Yamada AM Jr, Suzuki LC, Eiko Hashimoto MC, Cai S, Gouw - Soares S et al (2007) Bactericidal effect of malachite green and red laser on Actinobacillus actinomycetemcomitans. J Photochem Photobiol B 86 (1): 70–76.
[12] Goulart Rde C, Bolean M, Paulino Tde P, Thedei G Jr, Souza SL, Tedesco AC et al (2010) Photodynamic therapy in planktonic and biofilm cultures of Aggregatibacter actinomycetemcomitans. Photomed Laser Surg 28 (Suppl 1): S53–S60.
[13] Umeda M, Tsuno A, Okagami Y, Tsuchiya F, Izumi Y, Ishikawa I (2011) Bactericidal effects of a high - power, red light - emitting diode on two periodontopathic bacteria in antimicrobial photodynamic therapy in vitro. J Investig Clin Dent 2: 1–7.
[14] Chan Y, Lai CH (2003) Bactericidal effects of different laser wavelengths on periodontopathic germs in photodynamic therapy. Lasers Med Sci 18 (1): 51–55