Phototoxic effect of visible blue light on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* in patients with chronic periodontitis (An in-vitro study)

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ABSTRACT

Background: The aim of this study was to determine phototoxic effect of visible blue light on anaerobic periodontal pathogens namely *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Materials and methods: Strains of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were isolated from pockets of systemically healthy patients aged between 35-55 years old with pocket depths of 5-6 mm, the bacteria cultured on special blood Agar plates solid media, then subjected to visible blue light emitted from commercially available light cure devise (LED curing light); that emits blue light (400-500nm) of 1000mw energy at different periods of time exposures, then the CFU of each plate was measured by direct colony count with the aid of open CFU software after 48hours of anaerobic incubation.

Results: There was a decrease in CFU for both microorganisms as we proceeded from zero, 20, 40 and 60 seconds of blue light exposure.

Conclusion: there was a phototoxic effect for the visible blue light emitted from the light curing device against the anaerobic periodontal pathogens.

Key words: Blue light, CFU, anaerobic periodontal pathogen. (J Bagh Coll Dentistry 2015; 27(1):144-150).

INTRODUCTION

Chronic periodontitis is a quite common disease in adult patients characterized by pocket formation and/or recession while progressive loss of periodontal attachment occurs slowly to moderately local risk factors, e.g. bacterial plaque [1] .Wide array of microorganisms have been associated with periodontal disease, out of which *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) have been predominantly associated with periodontal diseases. The treatment of periodontal disease has always been inclined toward the disruption of these microbial floras either through mechanical therapy or by the use of antimicrobial agents [2].Hand instrumentation is still considered the gold standard and allows the sufficient cleaning of the periodontal pockets .Anatomical peculiarities like root curvatures or invaginations can make it difficult to remove bacterial deposits and biofilms completely from root surfaces by means of mechanical methods .Several treatment options are available to support the efficacy of instrumentation, for example the usage of local antibiotics or antimicrobials .or photodynamic therapy (PDT) [3].Different types of antibiotics have been used to avoid this obstacle. But another problem was noted as biofilm showed antibiotic-resistance mechanisms [4-6]

One of the problems that tackle the use of chemical agents is the failure in maintaining therapeutic concentrations in the targeted site and disruption of the oral microflora [7]**.** Photodynamic Therapy (PTD) thus was introduced to open a new path in treating periodontal diseases without being hindered by the obstacles and problems mentioned above, PTD contains three major components: visible light, a nontoxic photosensitizer, and oxygen [8]

The function of the exogenous Photosensitizers is to absorb the visible light that matches the wavelength of their peak absorption, then causing a photochemical mechanism that kills bacterial [9-11]. Interestingly, some bacteria can be eliminated without needing an exogenous photosensitizer. Among which are the Black-pigmented bacteria (BPB), it was assumed that excitation of their endogenous porphyrins will result in the death of bacteria [12]**.**

The BPB species found in the oral cavity can tolerate low concentrations of oxygen comparable to those levels in untreated human periodontal pockets although they are classified as anaerobes, these small amounts of oxygen render periodontal diseases susceptible to Photodynamic therapy (PDT) [13]**.**

Low-energy argon laser irradiation was proven to have phototoxic effects on *Porphyromonas*; *Prevotella* species [14,15].while similar effects were observed when utilizing visible light against *Porphyromonas gingivalis* and *Fusobacterium*

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nucleatum without an exogenous photosensitizer [16,17].

MATERIALS AND METHODS

Patient selection and sampling

Twelve systemically healthy patients of age range between 35-55 years old participated in this study, they had chronic periodontitis with at least one pocket of 5-6mm depth.

A piece of plaque from periodontal pocket was excavated by gracey curette without touching adjacent tissue.

Plaque sample was spread on blood agar solid media supplied with selective materials in the plates then plates were transported into an anaerobic jar with anaerobic gas pack incubated anaerobically for 72 hours.

After incubation, bacterial identification was based on (the microscopic appearance and colonial shape and size, gram stain, biochemical tests like catalyse, haemolytic capability, urease test, and antibiotic susceptibility tests).

Aggregatibacter actinomycetemcomitans colonies showed a convex white starry appearance with no black pigmentation **[Fig 1].** they were gram negative with rod shaped appearance under microscope, catalyse positive, coagulase negative , urease negative, had a Beta haemolytic activitiy and were resistant to Clindamycin and Metronidazole but sensitive to Kanamycin.

Porphyromonas gingivalis colonies were dull colored round convex colonies, clearly distinguished by the presense of black pigmentation **[Fig 2]**, they were gram negative with rod shaped (sometimes encapsulated) under microscope, catalyse negative, urease negative, had a weaker haemolytic activity, and susceptible to Clindamycin and Metronidazol Colonies were subcultered again on the same media anaerobically for 72 hours under the same condition,using the same method, to obtain pure cultures of both *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Fig 1: *Aggregatibacter actinomycetemcomitans* with its white starry shape

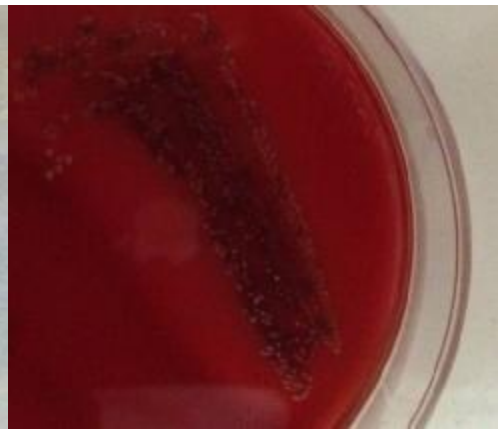


Fig 2: *Porphyromonas gingivalis* with black pigmentation clearly seen on plate

The application of light exposure using a serial dilution technique on microtiter plates:

After incubation period, a serial dilution procedure was performed for standardization of the amount of bacteria using 10^6 as bacterial initial concentration, and to decrease the numbers of colonies into a countable one. A standard volum of thioglycolate broth which is liquid media used to culture bacteria anarobically containing special reducing agents to be dispersed in each well of microtiter 96 well, (150 µl), then a single colony of each micro-organism was carefully chosen and mixed into the well of broth, from that well, we proceeded in dilution on 1:10 rate until we reached the 5^{th} dilution.

Four plates of enriched solid blood agar media were prepared for each bacteria; spreading broth taken from 5^{th} dilution well on each plates then exposed to different periods of light exposure, a light beam of blue light was directed on the plate.

starting from zero/seconds(no light exposure) for the first plate, then 20, 40, 60 for the 2^{nd} 3^{rd} and 4^{th} plate respectively; tip of the light cure devise is standardized with the center of light beam was directed towards the center of plate for all experiments. Then all plates were incubated anaerobically.

Counting CFU

The total colonial count was achieved using computerized program when needed named Open

CFU ver. 3.8.11, on day 13, the CFU's were counted by direct vision and with the use of "open CFU" software when conformation was needed. The plate that has no light exposure (zero second groups) for each micro-organism considered the control plate with which we compared the results of the remaining 3 plates. The whole procedure was repeated for each one of the 12 samples of patients who participated in the study.

RESULTS

A high significant statistical difference was observed in comparing the CFU count of *Aggregatibacter actinomycetemcomitans* at different periods of time of blue light exposure **[Table 1]** illustrates median value of CFU count of *Aggregatibacter actinomycetemcomitans* at different periods of blue light exposure, Showing a decrease in CFU count as we proceeded from zero to 60 seconds of blue light exposure **[Fig 3]** and **[Fig 4].**

In intergroup comparison **[Table 2]**, CFU count of *Aggregatibacter actinomycetemcomitans* at each period of light exposure time was compared to the CFU count at all the periods of light exposure.

Table 1: Median Value of *Aggregatibacter actinomycetemcomitans* CFU at different light exposure time

Time (Sec.)	Median	Mean Rank	X^2	d.f.	p-value	Significance
Control (zero)	358	37.67	20.61	3	0.000	HS
20	194	27.54				
40	142.5	19.75				
60	101	13.04				

***Kruskal-Wallis test was used**

Table 2: Intergroup comparison between *Aggregatibacter actinomycetemcomitans* CFU of each two groups of light exposure time

Time (Sec.)	Mann-Whitney U test	p-value
Control vs. 20 sec.	34	0.028 (S)
Control vs. 40 sec.	15	0.001 (HS)
Control vs. 60 sec.	9	0.000 (HS)
20 sec. vs. 40 sec.	46	0.133 (NS)
20 sec. vs. 60 sec.	23.5	0.005 (HS)
40 sec. vs. 60 sec.	46	0.133 (NS)

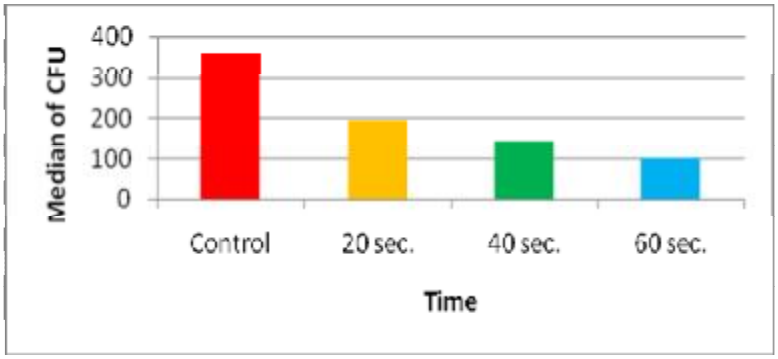


Fig. 3: Time of light exposure VS. The CFU of *Aggregatibacter actinomycetemcomitans*

A **B** **C** **D**

Fig. 4: decrease of *Aggregatibacter actinomycetemcomitans* CFU as we proceed from A: zero seconds of light exposure B: 20S, C:40S and D: 60S

There was a high significant statistical difference between: the control group (had no light exposure) and the 60 second group, control group and the 40 second group, the 20 second group and the 60 second of light exposure group**[Fig 5].**There was significant statistical difference between the control group and the 20 second group.There was no significant statistical difference between the 40 second group and the 60 second group, the 20 second group and the 40 second group.It's very obvious that the p-value decreased as the time difference increased between groups until reaching the highest significant value (0.00) when the difference was 60 seconds**.**

A significant statistical difference was observed in comparing the CFU count of *Porphyromonas gingivalis* at different periods of time of blue light exposure **[Table 3]** illustrates median value of CFU count of *Porphyromonas gingivalis* at different periods of blue light exposure, Showing a decrease in CFU; as we proceeded from zero to 60 seconds of blue light exposure **[Fig 6]** and **[Fig 7].**

In intergroup comparison **[Table 4]**, CFU of *Porphyromonas gingivalis* at each period of light exposure time was compared to the CFU at all the periods of light exposure. There was a high significant statistical difference between the control group (had no light exposure) and the 60 second group **[Fig 8].**

There was significant statistical difference between the 20 second group and the 60 second group, the 40 second group and the 60 second group.

There was no significant statistical difference between the control group and the 20 second group, the control group and the 40 second group, the 20 second group and the 40 second group.

P-value decreased as the time difference increased between groups reaching the highest significant value (0.003) when the difference was 60 seconds**.**

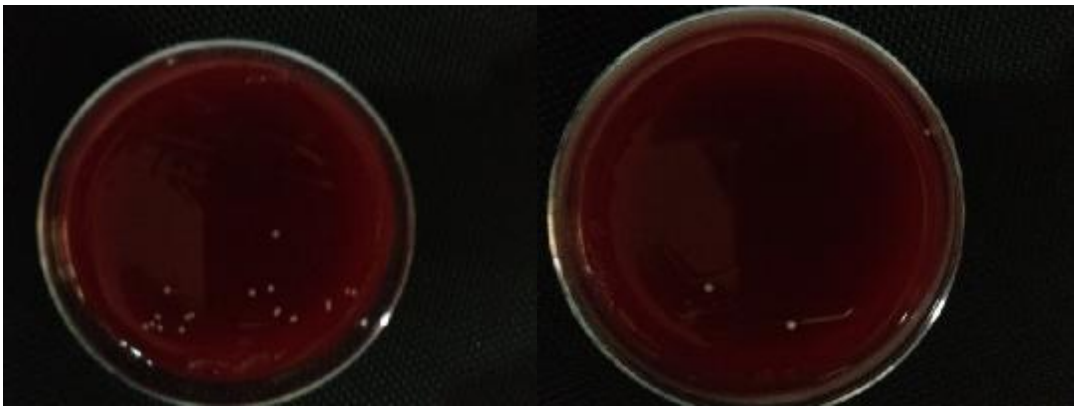


Fig 5: Difference of *Aggregatibacter actinomycetemcomitans* CFU between the (A) control plate (Zero light exposure) and (B) the 60 seconds exposure plate.

Table 3: Median Value of *Porphyromonas gingivalis* CFU at different light exposure time

Time (Sec.)	Median	Mean Rank	X^2	d.f.	p-value	Significance
Control (zero)	277.5	32.29	10.431	3	0.015	Sig
20	202.5	26.29				
40	177	25.21				
60	125	14.21				

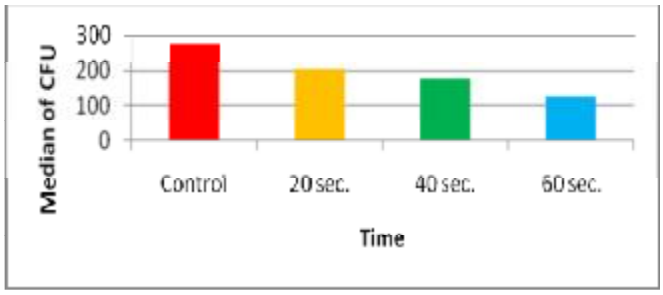
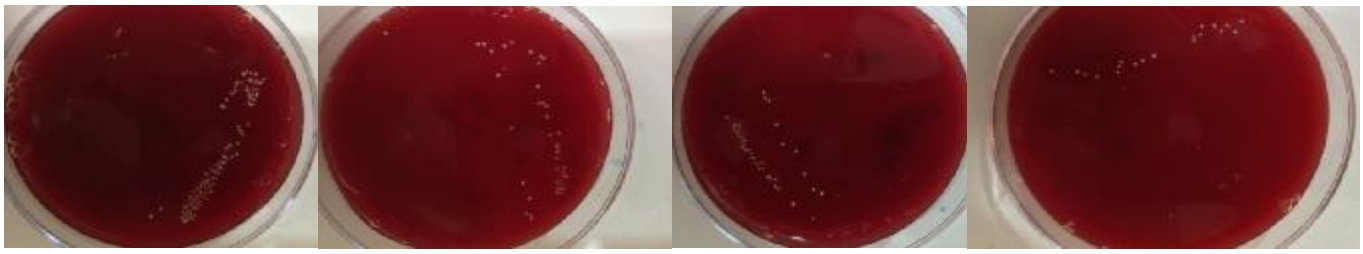


Fig. 6: Time of light exposure vs. The CFU Porphyromonas gingivalis



A **B** **C** **D**

Fig. 7: decrease of *Porphyromonas gingivalis* CFU as we proceed from A: zero seconds of light exposure through B: 20s, C: 40s and D: 60s seconds.

Table 4: Intergroup comparison between CFU of *Porphyromonas gingivalis* of each two groups of light exposure time

Time (Sec.)	Mann-Whitney U test	p-value
Control vs. 20 sec.	50	0.204 (NS)
Control vs. 40 sec.	52	0.248 (NS)
Control vs. 60 sec.	20.5	0.003 (HS)
20 sec. vs. 40 sec.	65.5	0.707 (NS)
20 sec. vs. 60 sec.	35	0.033 (S)
40 sec. vs. 60 sec.	37	0.043 (S)

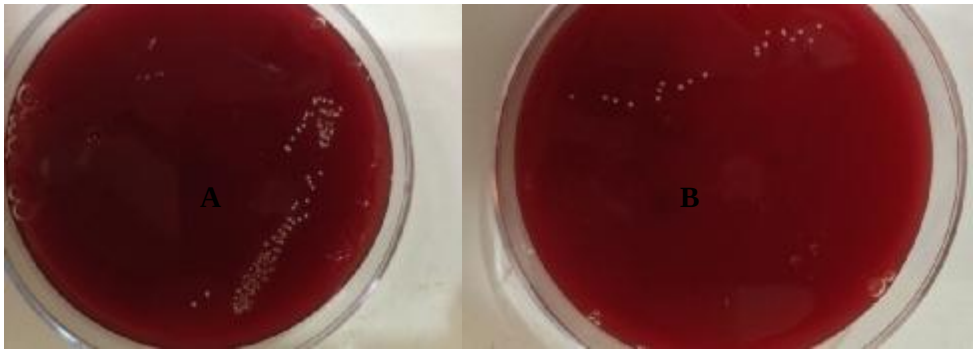


Fig 8: Difference of *Porphyromonas gingivalis* CFU between (A) the control plate (Zero light exposure) and (B) the 60 seconds exposure plate

DISCUSSION

Results regarding *Porphyromonas gingivalis* obtained from this research came in agreement with a study done by **Feuerstein et al.** who suggested a phototoxic effect of visible blue light on Gram negative anaerobic periodontal pathogens without use of exogenous photosensitizer [16].

Results regarding *Porphyromonas gingivalis* came in agreement also with a study done by **Hyun-Hwa Song et al.** but in disagreement with the same study as much as its concerned with *Aggregatibacter actinomycetemcomitans* results where he found no significant phototoxic effect of visible blue light against it, he found that there was a phototoxic effect of visible blue light emitted from a halogen light curing device source

on planktonic anaerobic periodontal pathogens, but suggested the use of exogenous photosensitizer if this method was to be used clinically, to increase the phototoxic effect[18].

A high significant statistical difference was observed in comparing the CFU of *Aggregatibacter actinomycetemcomitans* at different periods of time of blue light exposure , and there was a significant statistical difference was observed in comparing the CFU of *Porphyromonas gingivalis* at different periods of time of blue light exposure.This can be explained by the decrease of bacterial CFU of both *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* directly with the period of exposure to the curing blue light.

The decrease of bacterial CFU is explained by the killing ability of light and temperature against these bacteria, visible light (408-750 nm) has been found to be mutagenic and to cause metabolic and membrane damage to bacteria, oxidative stress occurs with reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radicals that damage proteins, DNA, lipid, and the cell membrane.

Light sources have considerably stronger effects with reactive oxygen radicals who occur in combined form with natural photosensitizers, such as humic acid or protoporfirin, it was also found that Enzyme synthesis such as Super Oxide Dismutase and catalyse have been shown to decrease with the effects of light independently [19].

The accumulative effect of light as time of exposure increases produces a decrease in the CFU count. In intergroup comparison of *Aggregatibacter actinomycetemcomitans* there was a high significant statistical difference between: the control group (had no light exposure) and the 60 second group, control group and the 40 second group, the 20 second group and the 60 second of light exposure group. There was significant statistical difference between the control group and the 20 second group. There was no significant statistical difference between the 40 second group and the 60 second group, the 20 second group and the 40 second group.

The intergroup comparison regarding the *Porphyromonas gingivalis*, there was a high significant statistical difference between the control group (had no light exposure) and the 60 second group, there was significant statistical difference between the 20 second group and the 60 second group, the 40 second group and the 60 second group, and there was no significant statistical difference between the control group and the 20 second group, the control group and the 40 second group, the 20 second group and the 40 second group.

This suggests clearly that the effect of blue light exposure increases as the time of exposure increases, whenever the difference of blue light exposure time between groups increases, the difference between CFU's was more significant, and the best results were obtained when there was a (60 seconds) difference, the results of comparison was high significant in both organisms.

As conclusion, there was a phototoxic effect for the visible blue light emitted from the light curing device against the anaerobic periodontal pathogens.

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