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RESEARCH ARTICLE

PHOTOTOXIC EFFECT OF VISIBLE BLUE LIGHT ON *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* AND *PORPHYROMONAS GINGIVALIS* ISOLATED FROM PATIENTS WITH CHRONIC PERIODENTITIS: AN *IN-VITRO* EXPERIMENTAL STUDY

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ABSTRACT
Chronic periodentitis 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. 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 objectives of this study were to determine phototoxic effect of visible blue light on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* clinical isolates from chronic periodentitis patients, and to study their antibiotic sensitivity against selected antibiotics. The test was carried out on 15 strains of *Aggregatibacter actinomycetemcomitans* and 15 strains of *Porphyromonas gingivalis* isolated from pockets of chronic periodentitis patients aged between 30-50 years old with pocket depths of 5-6 mm. The bacteria cultured, isolated, and identified by standard bacteriological methods, then subjected to visible blue light at different periods of time exposures. After light exposure, the bacterial killing rates were calculated from colony forming unit (CFU) counts after 48hours of anaerobic incubation. There was a decrease in CFU for both microorganisms as we proceeded from zero, 20, 40 and 60 seconds of blue light exposure. In conclusions, there was a phototoxic effect for the visible blue light emitted from the light curing device against the anaerobic periodontal pathogens and blue light exposure is effective in reducing periodontal pathogens. It is recommended that an adjunctive exogenous photosensitizer be used and that pathogens be exposed to visible light for clinical antimicrobial periodontal therapy.
Keywords: Anaerobic periodontal pathogen, blue light, CFU.

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INTRODUCTION

Periodontal diseases are complex, multifactorial, polymicrobial infections characterized by the destruction of tooth-supporting tissues. The disease begins as acute inflammation of the gingival tissue and untreated infections can progress to formation of teeth pockets, and eventually loss of teeth. According to the World Health Organization, periodontal disease affects 10–15% of adult population worldwide[1]. 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[2,3]. 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[4]. 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)[5]. Different types of antibiotics have been used to avoid this obstacle. But another problem was noted as biofilm showed antibiotic resistance mechanisms[6-8]. 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[9]. 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. As an innovative non-antibiotic approach, antimicrobial blue light (aBL) in the spectrum of 400–470 nm has demonstrated its intrinsic antimicrobial properties resulting from the presence of endogenous photosensitizing chromophores in pathogenic microbes. It is envisioned that microbes are less able to develop resistance to aBL than to traditional antibiotics, because of the multi-target characteristics of aBL[10]. In addition, it is well accepted that aBL is much less detrimental to host cells than UVC irradiation[11,12]. The objectives of this study were to determine phototoxic effect of visible blue light on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* clinical isolates from chronic periodentitis patients, and to study their antibiotic sensitivity against selected antibiotics.

MATERIALS AND METHODS

Patient selection and sampling

Fifteen systemically healthy patients of age range between 30-50 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**.** 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 presence of black pigmentation. 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 Metronidazole. Colonies were sub-cultured 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*.

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 volume of Thioglycollate broth which is liquid media used to culture bacteria anaerobically containing special reducing agents to be dispersed in each well of micro-titer 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 5th dilution. Four plates of enriched solid blood agar media were prepared for each bacteria; spreading broth taken from 5th 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 2nd, 3rd and 4th 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. The visible blue light emitted from commercially available light cure devise (LED curing light); that emits blue light (400-500nm) of 1000mw energy. After light exposure, the bacterial killing rates were calculated from colony forming unit (CFU) counts after 48hours of anaerobic incubation. The total colonial count was achieved on day 13; the CFU's was counted by direct vision. 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 15 samples of patients who participated in the study.

RESULTS

It is very obvious that the values of CFU decreased significantly ($p=0.001$) as the time of exposure difference increased between groups until reaching the highest value when the difference was 60 seconds**.** There was significance effect of visible blue light on the CFU of anaerobic periodontal pathogens *Aggregatibacter actinomycetemcomitans in-vitro* at different light exposure time. There was increase in the inhibition of growth in which the inhibition rates for 20 sec exposure, 40 sec exposure and 60 sec exposure were 41.2%, 54.2%, and 64.5% respectively. Also, there was decrease of Mean±SD of CFU as we proceed from A: zero seconds of light exposure, B: 20S, C: 40S and D: 60S [305.6±36.9 to 179.7±18.6 (20S), 140±15.9 (40S) and 108.6±13.8 (60S)]. In intergroup comparison CFU of the bacteria 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 (p=0.001) (Table 1, 2). Table 3and Table 4 shows the phototoxic effect of visible blue light on the CFU of anaerobic periodontal pathogens *Porphyromonas gingivalis* at different light exposure time. It is very obvious that the values of CFU decreased significantly (p<0.001) as the time of exposure difference increased between groups until reaching the highest value when the difference was 60 seconds**.** By comparing with zero exposure, the inhibition rates for 20 sec exposure, 40 sec exposure and 60 sec exposure were 22.04%, 35.4%, and 49.7% respectively. Also, there was decrease of Mean±SD of CFU as we proceed from A: zero seconds of light exposure, B: 20S, C: 40S and D: 60S (236.8+28.8 at zero time to 184.6±14.7, 153.1±15.4, and 119±9 respectively).

DISCUSSION

In the current study there was significance effect of visible blue light on the CFU of anaerobic periodontal pathogens *Aggregatibacter actinomycetemcomitans in-vitro* at different light exposure time in culture media in which the inhibition rates for 20 sec exposure, 40 sec exposure and 60 sec exposure were 41.2%, 54.2%, and 64.5% respectively in comparing with zero exposure time (Table1). Also the Mean±SD of CFU was significantly (p<0.05), decreased from 305.6+36.4 at zero time to 179.7±18.6 (20 sec), 140.1±15.9 (40 sec), and 108.6±13.8 (60 sec) respectively (Table 2). These results confirmed the toxic effect of visible blue light on *Aggregatibacter actinomycetemcomitans.* This effect can be explained by the fact that 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[13,14,15]. The current study results are similar to that reported by Henry *et al*. in which the visible blue light was proven to have phototoxic effects on *Porphyromonas*; *Prevotella* species[16,17]. Also similar effects were observed when utilizing visible light against *Porphyromonas gingivalis* and *Fusobacterium nucleatum* without an exogenous photosensitizer[18,19].

In addition, results regarding *Porphyromonas gingivalis* obtained from this research is 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[19]. 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 it's concerned with *Aggregatibacter actinomycetemcomitans* results where they found no significant phototoxic effect of visible blue light against *Aggregatibacter actinomycetemcomitans*. Also, they found that there was a phototoxic effect of visible blue light emitted from a halogen light curing device source on planktonic anaerobic periodontal pathogens[20].

A high significant statistical difference (p<0.05) 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. That's mean more exposure time leads to more bacterial death. 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. Also 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. As well light sources have considerably stronger effects with reactive oxygen radicals which occurs in combined form with natural photosensitizers, such as humic acid or protoporfirin. It was also found that Enzyme synthesis of bacteria such as Super Oxide Dismutase and catalyse of bacteria have been shown to decrease with the effects of light independently[21].

The current study results suggest 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, and 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. Furthermore, one of the advantage of photodynamic therapy (PDT) as visible blue light is its safety, as it be confirmed by Dai *et al*. a, b, Ramakrishnan *et al*. and Zhang *et al.* in which they showed that under certain exposures, no cytotoxic or genotoxic effects on relevant host cells[22-25]. In addition, no evidence of genotoxicity of visible blue light (aBL) was observed in mouse skin in vivo when exposed to the therapeutic aBL exposure for inactivating mature biofilms[26].

CONCLUSION

In conclusions, there was a phototoxic effect for the visible blue light emitted from the light curing device against the anaerobic periodontal pathogens and blue light exposure is effective in reducing periodontal pathogens. It is recommended that an adjunctive exogenous photo-sensitizer be used and that pathogens be exposed to visible light for clinical antimicrobial periodontal therapy.

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CONFLICT OF INTEREST

"No conflict of interest associated with this work".

AUTHOR'S CONTRIBUTION

This research work is part of MSc thesis. The candidate is the second author (AAA) who conducted the works and the experiments and wrote up the thesis. The corresponding author (HAA) supervised the experimental work, revised and edited the thesis draft and the manuscript. (MAA) was co-advisor of the work, and (AA) helped in dental works and laboratory works**.**

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Table 1: The phototoxic effect of visible blue light on the CFU of anaerobic periodontal pathogens *Aggregatibacter actinomycetemcomitans* at different light exposure time

CFU Values	Time of exposure (sec)			
	Zero	20 sec	40 sec	60 sec
Mean	305.6	179.7	140.1	108.6
Variance	1366.6	346	253.6	190.6
Standard division	36.9	18.6	15.9	13.8
Standard error	9.5	4.8	4.11	3.5
Minimum	208	140	102	80
Maximum	370	210	165	125
Median	305	180	143	110
Mode	300	190	130	100
Sum	4584	2696	2101	1629
student test	32	37.4	30.4	30.4
P value	<0.001	<0.001	<0.001	<0.001
Inhibition growth rate	Ref	41.2%	54.2%	64.5%

Table 2: The significance of the phototoxic effect of visible blue light on the mean ±SD CFU of anaerobic periodontal pathogens *Aggregatibacter actinomycetemcomitans* at different light exposure time

Time of exposure (sec)	CFU of tested bacteria Mean ±SD	X^2	P value
Control (Zero)	305.6±36.9	Reference	
20 sec	179.7±18.6	12.02	0.002
40 sec	140±15.9	16.2	0.0007
60 sec	108.6±13.8	19.79	0.0003

Table 3: The phototoxic effect of visible blue light on the CFU of anaerobic periodontal pathogens *Porphyromonas gingivalis* at different light exposure time

CFU Values	Time of exposure (sec)			
	Zero	20 sec	40 sec	60 sec
Mean	236.8	184.6	153	119
Variance	832.1	218	237.2	81
Standard division	28.8	14.7	15.4	9
Standard error	7.4	3.8	3.9	2.3
Minimum	200	168	130	100
Maximum	317	215	180	135
Median	230	185	153	120
Mode	210	170	130	120
Sum	3552	2769	2295	1786
student test	31.8	48.3	38.4	51.2
P value	<0.001	<0.001	<0.001	<0.001
Inhabitation growth rate	Ref	22.04%	35.4%	49.7%

Table 4: The significance of the phototoxic effect of visible blue light on the mean ±SD CFU of anaerobic periodontal pathogens *Porphyromonas gingivalis* at different light exposure time

Time of exposure (sec)	CFU of tested bacteria, Mean±SD	X^2	P value
Control (Zero)	236.8±28.8	Reference	
20 sec	184.6±14.7	6.3	0.01
40 sec	153±15.4	10.16	0.004
60 sec	119±9	15.5	0.001