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Research Article

Comparing the Effect of LAD (Light Activated Disinfectant), Chlorhexidine Gel, and Tetracycline on Bacteria Aggrigatibacter Actinomycetemcomitans (A.A)

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INTRODUCTION

The accumulation of biofilm on the surface of the teeth or gingival tooth surfaces leads to tooth decay or periodontal disease (1).Periodontitis is the most common disease in the oral cavity caused by mandatory gram-negative anaerobic bacteria andattachment loss and bone destruction and eventually may cause tooth loss (2).Aaas a major factor of Localized Aggressive periodontitis disease considered,the disease is characterized by rapid destruction of the tissues that support the teeth or 14% of 45-54 year old and 23% of 65-74 year-olds involved in the United States (3).Bacterial endocarditis is seen in cases of brain abscess and subcutaneous as an opportunistic pathogen isolated (4).The main approach for the treatment of periodontitis includesremoval by subgingival and supra-gingival plaque mechanical debridement (ie, non-surgical periodontal treatment) (5), which may be supplemented with antibiotic treatment (4). Many studies have been reported significant improvement in clinical and microbiological parameters for non-surgical periodontal treatment (6).However, none of the existing instrumentation techniques for the removal of subgingival bacteria and crime is not predictable (7). This limit may be attributed to various factors such as the complex anatomy of the teeth, periodontal pathogen invasion into surrounding soft tissues or re-colonization of periodontal pocket from other areas of the patient or shelters (Niches) inside the mouth (7).

However, articlesdescribes a series of antimicrobial substances for the use of topical to gels form, Biodegradable tablets and solution for washing, none of them have shown remarkable clinical results and there isno effective product (4).In addition, systemicadministration of the drug can have many effects such as bacterial resistance to digestive disorders, allergies, tetracycline absorbed by the teeth and bones, and so lack of alcohol intolerance (4).Drug inadequate concentrations within the sulcus fluid or biofilms can also be responsible for the lack of antimicrobial effect (3).They need to find new

approaches that do not have these problems in the periodontal treatment.Photodynamic therapy in the medical field in 1904 to disable the light-dependent cells, microorganisms or molecules entered, and based on the principle that a Photosensitizer (ie, a material can be activated by light) binds to the target cells, and can be activated by light of an appropriate wavelength (6).PDT is a successful technique for the treatment of many diseases, including abnormal cell growth, such as Cancer, rheumatoid arthritis, pathologic myopia, Vitiligo, muscle degeneration and atherosclerosis. Then success in application of PDT as anti-bacterial and anti-fungal materialwas observed (8).Photodynamic therapy for periodontal treatment has also been examined (9). Using a variety of dies photosensitizing (methylene blue or chlorine) (10), and by lasers with different wavelengths (11).

Since chlorhexidine and tetracycline, as the most common Adjunctive antimicrobials in the treatment of periodontitis are used. Therefore, this study aimed to compare the efficacy of chlorhexidine, tetracycline and photodynamic therapy on bacteria Aa was performed. For photodynamic therapy in this study, using a new combination Dye / laser is used.

MATERIALS AND METHODS

Isolation of bacterium

Ten patients (between 30 and 50 years of age) with active periodontal sites were treated with scaling and root planning (SRP). None of the patients were smokers, had implants or had received antibiotic therapy within the last six months. Tryptic Soy Serum Bacitracin Vancomycin agar (TSBV) was used as an enriched selective medium for the isolation of *Actinobacillusactinomycetemcomitans.* Samples were collected, using endodontic paper points, from periodontal sites of patients with chronic periodontitis (pocket depth 7 mm) and transferred in sterile vials containing 1 mL of BHI (Merck, Germany), Yeast extract 1% (Merck, Germany) and 10% Glycerol (Merck, Germany). The liquid culture was transferred to TSBV medium in culture plates and all of the plates were incubated at 37˚C for 72 h in an atmosphere of 5% CO2 in a CO2 incubator.

Preparation of specimens

Ninety freshly extracted, intact, adult, human, single-rooted, mature teeth with a single canal were collected and stored in sterile saline. Calculus and stains were removed from the root surface using an ultrasonic scaler (Cavitron, Dentsply Ltd, Weybridge UK).[11] After preparation of coronal two-thirds of all canals using Gates Glidden fi les number 4, 3 and 2, they were sequentially prepared within 1 mm apical end of the canal, using Hedström fi les (Antaeos, Munich, Germany) up to size 40. The canal was irrigated with physiologic saline after the use of each size fi le. To remove the smear layer that had developed on the canal wall as a result of the instrumentation, each canal was rinsed with 10% citric acid. Only teeth with closed apices tight for solutions were used in this study. All teeth were dried with paper points and weighed. The teeth were then stored in demethylated ethanol 70%. The prepared tooth was mounted in the lid of a bijou bottle. The assembled tooth, lid, and bottle were covered with aluminum foil and autoclaved at 121°C, for 15 min. The bottle was then aseptically fi lled with sterile brain heart infusion broth (BHI) (Merck, Poole, UK) so that the root was covered.

Experimental groups

Roots were randomly divided into three experimental groups. Bacterial suspension was prepared using a pure culture of this bacteriumstrain, grown in BHI broth medium. The inoculated medium was incubated at 37˚C for 24 hour. After 24 h, broth culture was inoculated into 8.5% normal saline solution to obtain an optical turbidity of 0.5 McFarland standard. Then 1ml of this the solution is inoculated in to the root canals in test tube for each of three groups. Then experimental groups were subjected by the following experimental treatment protocols:

Photodynamic therapy

Root canal infected with *A.a*was subjected to lethal photosensitization with Emoundo A.R.C

(Laser Gmbh Nurnberg Deutshland, Germany) with concentration g/mL and diode laser for 60 seconds. The irradiation source was a diode laser (fotoson CMS Dental, Denmark) with a total power of 200 mw/cm2. and 625 nm of wavelength.

Tetracycline gel

The canals were inoculated with tetracycline (Playstation Ron, Japan) for 5 min, removed with sterile paper points, and then subjected to PDT as described above.

Chlorhexidine* (CHLOSITE) *irrigation

Root canals were injected with Chlorhexidine (2% v/v) (Casalecchio di Reno Ialy) for 5 min, removed with sterile paper points, and irrigated with normal saline solution (.9% v/v).

Control groups

Controls consisted of no treatment (positive control) and without inoculation of bacterium (negative control) for three experimental groups.

Sampling procedures

Following all treatments, the liquid content of the root canal was carefully absorbed with paper points. Canals were filled with sterile 0.9% normal saline solution, and sample was absorbed with the frequent use of three paper points placed to the working length. Then by Curettes Samplingwas conducted,and both the Paper points and the Curette were transferred to tubes containing 1 ml of 0.9% normal saline solution and was vortexed for 1 minute. After 10-fold serial dilutions in saline, aliquots of 0.1ml were plated onto TSBV agar plates and incubated at 37°C for 48 hours. The colony forming units (CFUs) grown were counted. Data were analyzed by Mann-Whitney U test at 5% significance level. The significance level for all analyses was set at P <.05.

DATA ANALYSIS

For comparison, the data in different groups of ANOVA and compare pairs of groups, Tukey test will be used. Values composed colonies (CFU) Aggrigatibacter actinomycetemcomitans in various treatment groups were determined and reported. CFU values of Aggrigatibacter actinomycetemcomitans colonies after various antimicrobial treatments in general; using ANOVA analysis review and pairwise comparison of treatment groups both through Tukey test was conducted.

For statistical analysis SPSS software version, 0.18 was used. In this study, Type I error (α) is set to 0.05and if the p value (Type II error or β) equal to 0.05 or less than it obtained, the difference was deemed statistically significant.

Ethical Considerations

Since the research in the laboratory was conducted, where there were no particular problems in terms of ethical considerations.

FINDINGS

Based on the results, the average number of CFU after contact with tetracycline samples $10^5 \times 4.92$; in contact with chlorhexidine 1% of $10^5 \times 1.068$; after exposure to die indocyanine alone is $10^6 \times 3.25$; and after Photodynamic Therapy is equal to $10^4 \times 899.1$.After converting the numbers into logarithmic values, the average number of CFU after contact with tetracycline were 5.64 times; in contact with chlorhexidine 1% is 4.80; after exposure to Indocyanine die alone is 6.35, and after Photodynamic Therapy is 3.99.

In Table 1, statistics indicator Aggrigatibacter actinomycetemcomitans colonies value in therapeutic different groups and in Table 2 Aggrigatibacter actinomycetemcomitans colonies values based on log-transformed values, is shown.Accordingly, most of reduction in the bacterial population viability in treatment with Photodynamic Therapy was seen, and then, by treatment with tetracycline and chlorhexidine reduced bacterial colony counts Aggrigatibacter actinomycetemcomitans. Statistical analysis Anova showed statistically significant differences in term of the number of colonyforming bacteria treated with antimicrobial agents have been different ($p < 0.05$: in all groups).In addition, based on test results Tukey (thus pairwise comparison of CFU in different groups);difference in CFU of Aggrigatibacter actinomycetemcomitans bacteria

in samples of different groups after treatment was significant (statistically p <0.05).Reducing microbial load in Photodynamic Therapy group was significantly higher than tetracycline (p <0.100) and chlorhexidine (p = 0.001) and D (0.001p <), respectively.Reduction of microbial load in the chlorhexidine group was significantly higher than tetracycline (0.001p <) and Die (p <0.001).Moreover, the reduction of microbial load in the tetracycline group was significantly higher than Die (p =0.002).In Table 3, the results of ANOVA analysis of variance between the different groups, and in Table 4, Tukey test results on comparing different pairs of groups have shown.

Accordingly, PDT regime with Indocyanine green, was significantly more effective in removing bacteria from the root canals were from other regimes (p≤0.001) (Table 1)

Table 1. Values parts of composed Aggrigatibacter actinomycetemcomitans colonies after exposure to different antimicrobial treatments

Antibacterial		N	Minimum	Maximum	Mean	Std. Deviation
Tetracycline	Cfu	10	210000.00	830000.00	492000.0000	2.35126E5
	Valid N (listwise)	10				
CHLO site	Cfu	10	18000.00	360000.00	106800.0000	1.19878E5
	Valid N (listwise)	10				
Dye	Cfu	10	620000.00	6300000.00	3.2500E6	2.38836E6
	Valid N (listwise)	10				
Laser	Cfu	10	2200.00	73000.00	18990.0000	23331.78328
	Valid N (listwise)	10				

Table 2. The logarithmic values of the constituent units Aggrigatibacter actinomycetemcomitans colonies after exposure to different antimicrobial treatments

Descriptive Statistics						
Antibacterial		N	Minimum	Maximum	Mean	Std. Deviation
Tetracycline	log.cfu	10	5.32	5.92	5.6433	.22135
	Valid N (listwise)	10				
CHLO site	log.cfu	10	4.26	5.56	4.8071	.44774
	Valid N (listwise)	10				
Dye	log.cfu	10	5.79	6.80	6.3598	.41584
	Valid N (listwise)	10				
Laser	log.cfu	10	3.34	4.86	3.9970	.51640

Table 3. Results of ANOVA analysis of variance between different groups

log.cfu					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.431	3	10.477	60.819	.000
Within Groups	6.202	36	.172		
Total	37.633	39			

Table 4 Tukey test results between different groups (log.cfu)Tukey HSD

Tukey HSD								
(I) antibacterial		(J) antibacterial		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
dimension2	Tetracycline	dimension3	CHLO site	.83615*	.18562	.000	.3362	1.3361
			Dye	-.71653*	.18562	.002	-1.2164	-.2166
			Laser	1.64625*	.18562	.000	1.1463	2.1462
	CHLO site	dimension3	Tetracycline	-.83615*	.18562	.000	-1.3361	-.3362
			Dye	-1.55267*	.18562	.000	-2.0526	-1.0528
			Laser	.81010*	.18562	.001	.3102	1.3100
	Dye	dimension3	Tetracycline	.71653*	.18562	.002	.2166	1.2164
			CHLO site	1.55267*	.18562	.000	1.0528	2.0526
			Laser	2.36278*	.18562	.000	1.8629	2.8627
	Laser	dimension3	Tetracycline	-1.64625*	.18562	.000	-2.1462	-1.1463
			CHLO site	-.81010*	.18562	.001	-1.3100	-.3102
			Dye	-2.36278*	.18562	.000	-2.8627	-1.8629
*. The mean difference is significant at the 0.05 level.								

DISCUSSION

this study showed that photodynamic therapy by Indocyanine green and laser diode with a Wavelength of 810 nm acts effectively in reducing the amount of bacteria colonies Aggrigatibacter actinomycetemcomitans, as well as the antibacterial material used tetracycline and chlorhexidine is more effective in reducing the number of Aa colonies.Here we have a new composition Dye / laser representing a promising candidate as a adjunct to periodontal treatment.The laser used in this study is commonly used for soft tissue surgery in power 2W or higher is used.Under the conditions tested here (80-100 W / Cm)thelaser does not cut tissue but statistically significant killing bacteria is observed.Other studies from different diode lasers with a wavelength of 665 nm is used,Killing bacteria show using a longer exposure time (12, 13) (50-80 SEC), while laser light 810 nmalone may kill bacteria; on the condition that is long enough or have high powers. Present ICG increase kill bacteria in low power in a very short time.In this study, we use Indi cyanine green (ICG) as cardio green for using cardiovascular that is approved by FDA applications used; since its peak excitation wavelength corresponding to 810 nm generated by diode lasers commonly used for soft tissue surgery. ICG has low toxicity, high absorption in the near infrared and fast removal. According to a study (2004) Genina, ICG absorption peak near 810 nm, which is close to the maximum emission diode lasers on the market (15). In addition, ICG types can be built that allow accession by a carboxyl group (conjunction) to biologically active molecules of antibodies gel (17 and 16), and targeting specific bacteria and cellular components gives possible.Boehm study showed that ICG quickly by periodontal pathogens of an aqueous solution with low dose of ICG (ICG / 0.1 DMSO5-10 µM) is absorbed in the first 5 minutes, and more likely an incubation time of 1 minute with 10 Mµ lead to considerable withdraw ICG (11).It also showed that the harvest for specific periodontal bacteria, as a gram-negative bacteria (E.Coli) and gingival cells cultured values ICG absorb ten times less. This significant increase in the absorption of Aa and Pg is killed and the survival rate after laser irradiation with energy 400 W / Cm2 reached to 0.1 percent. (11)There are several theories about the need to pre-incubation with Die about Die in different die and various microorganisms. Because PS antimicrobial activity against Gram-negative bacteria is less than Gram-positive bacteria (16), contact die before exposure to increase the probability of interaction PS with cell wall Aa is required.In Die such as Rose Bengal, 30 minutes contact to Die before the effective time exposure to disable Aa for all concentrations is used. However, according to the study ICG Bohem quickly removed by periodontal pathogens in the first 5 minutes and most likely an incubation time of 1 minutes to 10 µMpicked up considerable ICG, this feature is that the benefits of this material (11). As a result , this study, incubation period of 5 minutes was used for die and photodynamic therapy.Many studies have shown that oral cavity bacteria that grow in Planktonic environment are sensitive to PDT.Since the microorganisms is responsible for oral diseases are organized in the form of biofilms that some of its characteristics is different from Planktonic growth, such as extracellular polymeric substances, different combinations of cell wall growth, metabolic activity, gene expression (18). Moreover, higher resistance bacteria in biofilms to microbial antibiotics materials; further studies should be performed to determine whether the combination of laser treatment by ICG significant clinical effects of biofilms in human periodontal pocket or not.The use of chlorhexidine in the present study at a concentration of 2% was carried out; according to study to evaluate the different concentrations of dissolved and different times (5-60 min) against various strains of microorganisms was chlorhexidine (19). It was found that the concentration of 2% solution in the shortest time back had the highest antimicrobial activity (20). According to some research, chlorhexidine hasnot significant effects on the stability of species Periodontopathogenic.In this study, chlorhexidine

significantly reduced in the Aacolonies, and also in reducing bacteria was more effective than tetracycline.

In this study, tetracycline than chlorhexidine and photodynamic therapy was less effective in reducing the amount of bacteria Aa, and given the likely increase in resistance of bacterial species that have been reported in clinical studies with this material and problems in its application in the clinic. It seems PDT, as a new approach to the treatment Adjunctive periodontal, can be a viable alternative to older methods.Despite the importance of laboratory studies to evaluate the performance of different treatments, care must be taken generalizability of the results of these studies in clinical settings and mouth disease is facing several problems. For application materials and conduct clinical processes carried out in the laboratory environment easily and it is also easily controlled confounding variables, while many intervening variables in a person's mouth, such as factors related to dentist and the patient who is very difficult to control them. In this respect, perhaps with further clinical trials identify the benefits of Photodynamic Therapy treatments.

CONCLUSION

Based on the results of this study on the effects of photodynamic therapy with ICG and laser diodes on Aggrigatibacter actinomycetemcomitans bacteria it seems that the photodynamic therapy with sensitive material Indocyanine green can be used as a method to increase the clearance of microbial components in periodontal treatment used.

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