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A paper extracted from Master thesis entitled (The antibacterial Potentiality of Photo-activated Disinfection Versus Chemical Disinfection on Carious Dentin).

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The Antibacterial Potentiality of Photo-activated Disinfection Versus a Chemical Disinfection Solution on Carious Dentin

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ABSTRACT

Aim: This study was conducted to assess the effect of different variables on antibacterial potential of photo-activated disinfection versus the effect of a chemical disinfection solution on carious dentin. **Material and Methods:** Seventy freshly extracted teeth were selected, All soft caries was removed by sterile excavator and specimens were weighted by five digit microbalance to be in range of 6-10 mg, Specimen divided into three groups: Group **A1**: disinfected with phot-activated disinfection (PAD) and divided into four subgroups (no=10) according to power& application time, Subgroup1(P1T1 : delivery power was 100mW, application time was 120 sec) Subgroup2 (P1T2: delivery power was 50mW application time was 30 sec) Subgroup3 (P2T1: delivery power was 100mW application time was 120 sec) Subgroup4 (P2T2: delivery power was 50mW,application time was 30 sec).Group **A2**: disinfected with chemical disinfectant (chlorohexidine scrub 2%).Group **A3**: disinfected with laser beam only & divided into two subgroups **P1T1,P2T2**. The specimens were plated & duplicated on blood agar then incubated in anaerobic gas pack jar at 37C for 7 days. **Results:** A statistically significant difference was found among PAD, chlorohexidine, and laser groups in the percent decrease in the total bacterial count ($P \leq 0.05$). The greatest mean percent decrease was recorded in the **PAD P1T1** followed by the chlorohexidine group, then the **laser P1T1**. **Conclusion:** The combination of toluidine blue O (TBO) and diode laser could be effective in reducing the bacterial viability. Chlorohexidine as chemical disinfectant was equally effective to photo-activated disinfectant with high delivery power and long contact time in reducing the total bacterial count.

INTRODUCTION

Caries is an infectious bacterial biofilm disease which is expressed in a predominantly pathologic oral environment. The purpose of caries control would better focus on diagnosis and elimination of the main contributing factors, which are microorganisms, not only the treatment of the lesion itself⁽¹⁾. Any leftover of bacterial remnants during and after the cavity preparation pose one of the major problem in restorative

KEYWORDS

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Chlorohexidine,
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dentistry, where failure to achieve complete serialization of the cavity preparation can lead not only to microleakage, pulp sensitivity, pulpal inflammation, but also to secondary caries necessitating replacement of the restoration ⁽²⁾. Accordingly, the application of disinfectant after cavity preparation and before tooth restoration is gaining acceptance. Unfortunately, microorganisms that irreversibly attached to a surface exhibit distinctive phenotypic properties and tend to be far more resistant to antimicrobial agents. Therefore, major research efforts directed towards discovering alternative antibacterial therapeutics to which bacteria would not develop resistance ⁽³⁻⁴⁾.

An alternative strategy to treat carious lesion would be killing the causative microorganism in situ. Photodynamic therapy (PDT) may constitute a suitable process to combat both biofilm and antimicrobial-related resistance. PDT is a treatment modality which employs the use of a photoactive dye, called a photosensitizer (PS) that is taken up into cells and is irradiated with light of an appropriate wavelength. This may result in cell death through the production of active oxygen species ⁽⁵⁾. The advantages of photoactivated disinfection over conventional antimicrobial approaches include simple delivery at the exact target area, little likelihood to develop microbial resistance, non-invasive nature, repeatability and high selectivity ⁽⁶⁾.

In an early study, suspensions of the cariogenic bacteria *S. mutans*, *S. sobrinus*, *Lactobacillus casei* and *Actinomyces viscosus* were exposed to light from a 7.3 mW helium neon laser in the presence of toluidine blue O (TBO) with different exposure times and different concentrations of the dye. *S. mutans*, *S. sobrinus* and *A. viscosus* were killed after a minimum exposure time of 15 s with a dye concentration of 100 µg/ml. However, when the same concentration of dye was used, *L. casei* was killed only after exposure to the laser light for at least 45 s. At a TBO concentration of 50 µg/ml, killing was observed with *S. mutans*, *S. sobrinus* and *A. viscosus* after exposure for 30 s. When sensitized by

TBO 25 µg/ml, *S. mutans* required an exposure time of only 30 s for a bactericidal effect to be detected whereas the other organisms required 60 s, indicating that *S. mutans* was more sensitive to the laser light than others. They concluded that neither the dye nor the laser light alone had any demonstrable effect on the viability of the organism ⁽⁷⁾.

The susceptibility to photo-activated disinfection (PAD) of *Streptococcus mutans* has been determined when the organism was present in a collagen matrix – an environment similar to that which would exist within a carious tooth. In addition, the susceptibility to (PAD) of bacteria present in carious human teeth was also determined. Light was delivered to the collagen and teeth using a system comprising a 0.8 –mm diameter isotropic tip emitting light at 633 + 2 nm. A single concentration of TBO (10 µg/ml) was used with both collagen and dentin. Two contact times 30 and 180 s, the effect of energy doses from 10.8 to 14.4 j on the kills attained was assessed by determining the number of surviving viable bacteria. In carious dentin, two contact times, 30 and 60 s and energy 4.8 j were used. The result showed that PAD can achieve appreciable kills of oral bacteria, including *S. mutans*, when the organism are embedded in a collagen gel or are present in carious teeth ⁽⁸⁾.

The antimicrobial effect of photodynamic therapy using toluidine blue O, in combination with either a helium-neon (HeNe) laser or light emitting diode (LED) with energy densities between 49 and 294 J/cm² was reported on the viability of streptococcus mutans biofilm. Significant decreases in the viability of *S. mutans* biofilms were only observed when biofilms were exposed to both TBO and light, when reductions in viability of up to 99.99% were observed with both light sources. It has been concluded that the bactericidal effect was light dose-dependent and that older biofilms were less susceptible to photodynamic therapy ⁽⁹⁾.

Moreover, another study assessed the clinical effect of photodynamic therapy (PDT) in the decontamination of the deep dentin of deciduous molars submitted to partial removal of carious tissue.

After cavity preparation, dentin samples were taken from the pulp wall of nineteen deciduous molars before and after PDT application. Remaining dentin was treated with 0.01% methylene blue dye followed by irradiation with an In GaAlP diode laser (λ – 660 nm; 40 mW; 120 J/cm²; 120 s). Dentin samples were microbiologically assessed for the enumeration of total microorganisms. There was no significant difference in the number of colony-forming units (CFU) for any of the microorganisms assessed. Photodynamic therapy using 0.01% methylene blue dye at a dosimetry of 120 J/cm² would not be a viable clinical alternative to reduce bacterial contamination in deep dentin ⁽¹⁰⁾.

The purpose of the present study was carried out to assess the effect of different variables on antibacterial potential of photo-activated disinfection versus the effect of a chemical disinfection solution on carious dentin.

MATERIALS AND METHODS

Seventy freshly extracted permanent mandibular molar with closed apices were selected. After extraction, each tooth was placed in a screw-capped microtube containing 2ml of thioglycolate broth as an anaerobic transporting medium, immediately sealed and carried out to the microbiology laboratory at the Faculty of Medicine Al-Azhar University within 1 h of extraction. All teeth were transferred from the microtubes to a sterile pad. Within each tooth, all soften carious tissues was removed using new sterile hand excavators and then divided into two approximately equal halves. Each was placed in a sterile preweighted Epindorff vial which was reweighted using a five digit microbalance. One-half of each tooth was not treated with any disinfecting agent and served as a control, while the other-half was treated with one of disinfecting agent either chemical or a photo-activated disinfectant. Accordingly, each tooth served as its own control. Each specimen weight ranged from 6-10 mg to standardize all specimens ⁽⁸⁾.

The specimens were randomly divided into three groups according to the disinfectant used: **Group A1:** Specimens were disinfected with photo-activated disinfection (PAD) (n= 40). The specimens were subdivided into four subgroups (10 specimens each) according to the delivery power and application time used to irradiate the carious tissues. **Subgroup 1(P1T1):** The delivery power was 100 mw with application time 120 seconds. **Subgroup 2 (P1T2):** The delivery power was 100 mw with contact time 30 seconds. **Subgroup 3 (P2T1):** The delivery power was 50 mw with application time 120 seconds. **Subgroup 4 (P2T2):** The delivery power was 50 mw with application time 30 seconds. **Group A2:** Specimens were disinfected with chemical disinfection (2% chlorhexidine gluconate) (n= 10). **Group A3:** Specimens were disinfected with laser beam without any disinfecting agent (n= 20). **Subgroup 1(P1T1):** The delivery power was 100 mw with application time 120 seconds. **Subgroup 2 (P2T2):** The delivery power was 50 mw with application time 30 seconds.

Application of disinfecting agent:

Group A1: photo-activated disinfection (PAD)

Specimens were sensitized with Toluidine blue O (TBO) for 2 min. The light source used for PAD was a diode laser delivered through EPIC BIO-LASE which produces light with a wavelength of 940 nm. The light was directed through a fiber optic cable with a 9.5 mm focal spot and maintained at a distance 2 mm.

Group A2: chemical disinfection: 2% chlorhexidine cavity disinfectant (Consepsis Ultradent) was applied. Consepsis solution consists of chlorhexidine gluconate with a pH of 6.0. The disinfectant stayed in contact with each specimen for 30 seconds.

Group A3: Laser : The specimens were irradiated with diode laser as mentioned previously in group A1 and not treated with any disinfecting agent, accordingly served as control group.

Microbiology procedures: In photoactivated groups (A1 and A3), the tip of the device was removed from vials and three glass beads (0.1mm in diameter) was placed in the tubes to detach the bacterial cells. The specimens were plated and duplicated on blood agar to determine total microorganism count. The plates were incubated in anaerobic jar (Gas pack System) under anaerobic conditions at 37°C for 7 days. The total microbial count per 1ml was determined by measurement of the number of colony forming unit (CFU) on each plate, Identification was based on colony morphology and Gram stain reaction.

Statistical analysis: Data were explored for normality using Kolmogorov-Smirnov tests. All data were normally distributed (parametric data), so paired-t test was used to compare before and after values within each group. ANOVA (Analysis of Variance) was used for comparison of mean values among the four photo-activated groups, where the significance level was set at $P \leq 0.05$. Tukey's test was used for pair-wise comparison between means when ANOVA was significant.

RESULTS

1- Comparison between pre and posttreatment total bacterial count within PAD, chlorhexidine and laser groups:(Table 1)(Figur 1)

In all groups, Paired-t test revealed that the mean bacterial count significantly decreased after treatment ($P \leq 0.05$). ANOVA test showed a statistically significant difference among PAD, chlorohexidine, and laser groups in the decrease in the total bacterial count ($P \leq 0.05$). The greatest mean decrease was recorded in the PAD P1T1 (delivery power was 100mW, application time was 120 sec with TBO) subgroup, followed by the chlorohexidine group, then the laser P1T1 (delivery power was 100mW, application time was 120 sec without TBO) and PAD P1T2 (delivery power was 100mW, application time was 30 sec with TBO) group. On the other hand, the lowest mean decrease in bacterial count was noted in laser P2T2 (delivery power was 50mW; application time was 30 sec without TBO).

Table (1): Mean values of total bacterial count ($\times 10^9$) in PAD, chlorhexidine and laser groups

	PAD								Chlorohexidine		Laser			
	P1T1		P1T2		P2T1		P2T2				P1T1		P2T2	
	pretreatment	posttreatment	pretreatment	posttreatment	pretreatment	posttreatment	pretreatment	posttreatment	pretreatment	posttreatment	pretreatment	posttreatment	pretreatment	posttreatment
Mean	4.746	1.97	2.94	1.48	3.21	1.83	5.17	3.59	4.95	2.44	4.146	2.269	5.076	3.259
SD	1.96	0.86	1.01	0.23	1.03	0.59	2.45	2.18	2.04	1.75	2.365	1.08	1.765	1.04
Min	2.5	1.03	1.63	1.13	1.50	1.07	1.60	0.80	1.40	0.20	1.500	0.600	1.370	0.809
Max	7.2	3.25	4.30	1.80	4.45	2.85	8.60	6.90	7.68	5.20	7.200	4.400	6.700	5.600
t value	-6.46		-4.47		-5.81		-11.16		-6.18		2.28		-4.67	
P value	0.000116*		0.004201*		0.001139*		<0.00001*		0.000105*		0.0348*		0.00343*	

* Significant at $P \leq 0.0$

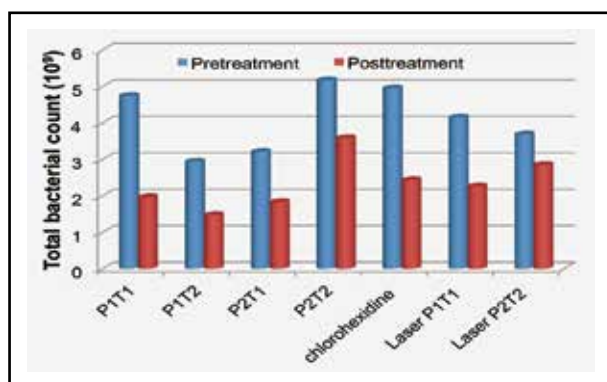


Fig. (1) Column chart showing mean values pre&post treatment in PAD, chlorhexidine and laser groups.

II-Comparison of percent change in total bacterial count among PAD, chlorhexidine and laser groups :(Table 2),(figure 2)

ANOVA test showed a statistically significant difference among PAD, chlorhexidine, and laser groups in the percent decrease in the total bacterial count ($P \leq 0.05$). The greatest mean percent decrease was recorded in the **PAD P1T1** (delivery power was 100mW, application time was 120 sec with TBO) subgroup, followed by the chlorhexidine group, then the **laser P1T1** (delivery power was 100mW, application time was 120 sec without TBO) and **PAD P1T2** (delivery power was 100mW, application time was 30 sec with TBO) group. On the other hand, the lowest mean percent decrease in bacterial count was noted in **laser P2T2** (delivery power was 50mW; application time was 30 sec without TBO).

Table (2): Percent decrease in bacterial count after treatment in all groups

	Percent decrease in bacterial count						
	PAD				Chlorohexidine	Laser	
	P1T1	P1T2	P2T1	P2T2		P1T1	P2T2
Mean	-57.59 ^a	-45.27 ^{ab}	-41.53 ^b	-35.29 ^b	-55.55 ^a	-47.81 ^{ab}	-22.25 ^c
SD	9.8	18.01	11.22	13.58	22.44	14.67	11.85
Min	-43.0818	-11.66	-28.67	-19.77	-14.75	-19.11	-9.03-
Max	-76.8254	-61.4	-56.4	-61.09	-85.71	-60	43.53
F value	32.67						
P value	<0.0001*						

*significant at $P \leq 0.05$.

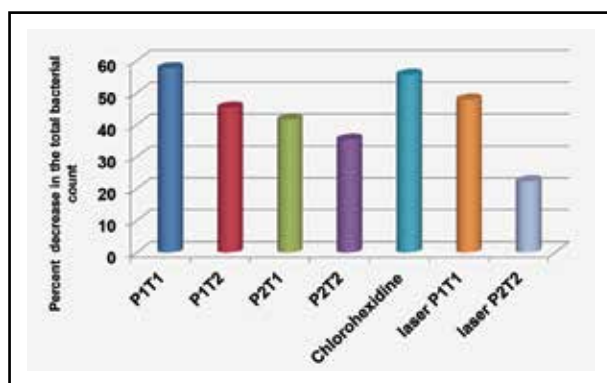


Fig. (2) Column chart showing mean percent decrease in total bacterial count after treatment in all groups.

DISCUSSION

The principal objective of caries removal is to eliminate the infected tissues and microorganisms that may be protected by the dentinal structure and can cause a persistent inflammation and treatment failure. However, the caries treatment procedures used presently not always assuredly eliminates all of the microorganisms in residual tissues. Therefore, there is a pressing need for strategies that are capable of inactivating pathogens. One possible option could be the use of antimicrobial photodynamic therapy (PAD), whereby the lethal effect of PAD is

based on the principle that visible light activates a photosensitizer (PS), leading to the formation of reactive oxygen species, which induce phototoxicity immediately during illumination ⁽¹¹⁾.

Chlorohexidine chemical disinfectant was used which is a cationic broad-spectrum antimicrobial agent that has been widely studied and proven effective in controlling dental biofilm. This effectiveness is directly related to a property denominated substantivity by which the molecule remains adhered to tissues and has antibacterial action for up to 12 hours ⁽¹²⁾.

Phenothiazinium derivatives have been employed as photosensitizers due to their strong absorption in the red spectral region and they are capable of inactivating both gram positive and gram negative bacteria. Toluidine blue O (TBO) photosensitizer which was used in this study belongs to phenothiazinium dyes that composed of three ring π -system with attached auxochromic side groups. They are single positively charged dyes and their singlet oxygen quantum is around 0.5 which is considered high ⁽¹³⁾.

Diode laser was used in the present study which has a resonant wavelength absorption band, less portable and low in cost ⁽¹⁴⁾. Diode laser has different mechanisms of action regarding its antibacterial effect which include: thermal and photodisruptive effect that are considered the principal reasons for the laser to eliminate the bacteria. Lethal damage include destruction of the cell wall integrity and possibly the denature of protein. The damage of the cell wall will cease the cell growth and successive cell lysis. At the same time, the cellular protein is highly sensitive to the thermal changes ⁽¹⁵⁾.

The results of the present study showed that the mean total bacterial count significantly decreased after treatment with all PAD subgroups ($P \leq 0.05$). However, there was a statistically significant difference among **P1T1** (power was 100mW, time was 120 sec with TBO), **P1T2** (power was 100mW, time was 30 sec with TBO), **P2T1** (power was 50mW, time was 120 sec with TBO) and **P2T2** (power was 50mW, time

was 30 sec with TBO) in the percent decrease in the total bacterial count ($P \leq 0.05$), where Tukey post hoc test revealed that, treatment with PAD (high delivery power 100mW and long application time 120 sec) (**P1T1**) showed the greatest percent decrease in the total bacterial count among the four PAD subgroups.

This may be attributed to that when the power of laser was increased, the disinfection effect increased ⁽⁸⁾, where the subgroups with 50 mW output power (**P2T1**,**P2T2**) had slightly inferior results regarding elimination of microorganisms compared to the subgroups activated by 100 mW output power (**P1T1**,**P1T2**).

The results of the present study was in accordance with several studies which reported that complete eradication of bacteria was achieved only through high-power irradiation of diode laser ranges from 0.5-15 W ⁽¹⁶⁾ and 980-nm diode laser achieved the maximum bacterial reduction when the amount of power delivered was increased ⁽¹⁷⁾. Moreover, it has been showed that using diode laser with 1.3W output power was more effective in streptococcus mutans reduction compared to diode laser with 1 W output power ⁽¹¹⁾.

The results of the present study showed that the subgroups with long application time 120 sec (**P1T1**) and (**P2T1**) had superior results regarding elimination of microorganisms compared to the subgroups with short application time 30 sec (**P1T2**) and (**P2T2**) respectively. This in accordance with a study which assumed that, increasing the contact time for 60 s allowed 400- μ penetration of the dye, which significantly increased the bacterial kill compared to those obtained for the 30 s application time ⁽¹⁸⁾.

The superior results of **PAD P1T1** (delivery power was 100mW, application time was 120 sec with TBO) subgroup may be attributed to that upon irradiation with light of an appropriate wavelength; the photosensitizer undergoes transition from low-energy-level "ground state" to a higher-energy "triplet state." This triplet-state sensitizer can react with biomolecules to produce free radicals and radical ions or with molecular oxygen to produce singlet

oxygen. This can cause oxidation of cellular constituents such as plasma membranes and DNA, resulting in cell death ^(5, 19).

CONCLUSION

Under the limitations of this study, the following can be concluded:

1. The combination of toluidine blue O (TBO) and diode laser could be effective in reducing the bacterial viability.
2. The delivery power and application time of laser were important parameters in the reduction of total bacterial count.
3. The uses of diode laser in the absence of toluidine blue O (TBO) was less effective than the combination of diode laser and TBO in reducing the bacterial count.
4. Chlorohexidine as chemical disinfectant was equally effective to photo-activated disinfectant with high delivery power and long contact time in reducing the total bacterial count.

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