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MICROBIOLOGICAL ASSESSMENT OF DIODE LASER IN COMPARISON TO CHEMICAL DISINFECTION WITH SELECTIVE CARIES REMOVAL

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KEYWORDS

Cavity disinfection, Chlorhexidine, Diode laser, Selective caries removal.

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ABSTRACT

Introduction: The treatment of dental caries has shifted dramatically in recent years as our comprehension of the caries process has increased. Diode laser has revealed favorable outcomes in cavity disinfection and stimulating reparative dentin formation. Therefore, the current study might be of value. **Aim:** This study was conducted to assess the antibacterial effect of Diode laser in comparison to chemical disinfection with partial caries removal. **Patients and Methods:** This randomized controlled clinical trial was carried out on 30 patients. The patients were randomly divided into 3 equal groups according to the method of cavity disinfection; group A_1 (application of Diode laser with 0.5 watt), group A_2 (application of Diode laser with 0.1 watt) and group A_3 (using 2% Chlorhexidine as Chemical disinfectant). Cavity preparation was first performed, and before the final restoration was placed, dentin specimens were taken from the cavity before and after application of disinfectant, then cultured on three types of selective media; Blood agar for total viable count, Mitis salivurias agar for Streptococcus mutans, and Rogosa agar medium for Lactobacilli. Microbiological data were statistically analyzed to assess the antibacterial effect of diode laser and chemical disinfection. **Results:** Bacterial count significantly decreased in all groups after application of intervention. **Conclusions:** Within the limitations of the current study, it can be concluded that Diode laser can show promising results in terms of cavity disinfection and management of deep caries lesions. In addition, Chlorhexidine is as effective as diode laser in cavity disinfection.

INTRODUCTION

The treatment of dental caries has shifted dramatically in recent years as our comprehension of the caries process has increased [(1)]. Earlier treatment modalities aimed for complete nonselective removal till reaching hard dentin, in order to prevent further caries recurrence and treatment failure[(2)].

However, such approach leads to unnecessary pulpal exposures, necessitating additional procedures such as pulp capping or even endodontic treatment[(3,4)]. Recent modalities have been addressed in treating dental caries, where only soft and infected dentin is removed[(4)]. These selective treatment approaches include step wise excavation and partial caries removal [(5)].

Stepwise excavation is considered to be a conservative treatment option in management of deep caries. It is a two-step technique, remaining carious soft dentin on the pulpal floor is left at the first visit and a temporary restoration is placed. At the second visit, the cavity is reentered, and the remaining soft dentin is removed. This technique is based on the assumption that in the first step cariogenic bacteria is reduced, and remineralization process is promoted with subsequent arrest of the carious lesion [(5)].

Although this technique revealed high success rates, it requires two visits, which add more cost and effort, furthermore it raises the risk of pulp exposures [(6)].Therefore, less invasive approach called “partial caries removal” or “selective caries removal” started to gain popularity [(7)].

Previous studies claimed that caries process becomes arrested in the remaining contaminated dentin, as cariogenic bacteria become nonviable due to absence of the substrate [(8)]. Despite these, there are very few studies on selective removal of soft dentin with long follow up periods [(9)]. In addition, it couldn’t be truly stated whether the residual bacteria or their byproducts would be damaging to the pulp or not [(10)]. As there is still controversy regarding the fate of microorganisms, some studies claimed that such bacteria could still persist under the restoration affecting the success of the treatment [(11)].

Therefore, clinically efficient adjunctive methods for disinfecting the remaining bacterially infected dentin may indeed be essential. Chlorhexidine 2% has been used for this purpose and has been appeared to be compelling at eradicating remaining cariogenic bacteria [(12)]. This is related to its chemical charge, since it has strong cationic properties, accounting for its tenacious potential and sustained antibacterial effect [(13)].

Application of lasers has extensively spread in the field of dentistry since the mid1990s. Among several types of lasers, Diode laser has shown some unique characteristics such as being compact, low in cost compared to other lasers, smaller in size, and acquires versatile additions [(1)].

Diode laser has revealed outstanding outcomes in root canal disinfection, surpassing the effect range of any other chemical disinfectant [(14)]. Also a recent study stated that Diode laser can be a safe alternative in cavity disinfection, since its temperature rise is far below the critical threshold of the pulp [(15)].

However, limited studies are available regarding the necessity and efficacy of Diode laser in cavity disinfection. Therefore, a clinical study assessing the antibacterial effect of Diode laser application versus chemical disinfection was thought to be of value.

PATIENTS AND METHODS

I.1. Study design

After the approval of the Research Ethical Committee (REC) of the Faculty of Dentistry, Suez Canal University with approval NO. #116/2018, this randomized controlled clinical trial was carried out on 30 patients selected from the Operative Department clinics in the Faculty of Dentistry, Suez Canal University, with an age range of 18 -50 years. One tooth per each patient was treated with a total number of 30 teeth. This trial was reported following the Consolidated Standards of Reporting Trials (CONSORT) guidelines to ensure transparent and complete reporting [(16)].

2. Sample size calculation

The total sample size was determined using power analysis for a Chi-square test for comparison between three groups. Calculation utilized success rate as the primary outcome based upon the results of Uday Mohan et al (2016) [1]. The effect size (w) was 0.75, using alpha (α) level of 0.05 (5%) and Beta (β) level of 0.10 (10%) i.e. power = 90%; the minimum estimated sample size was a total of 26 subjects. The number was increased to 30 subjects (30 teeth). So, each group included 10 subjects (10 teeth). Sample size calculation was performed using G*Power software (Version 3.1.9.2; Heinrich-Heine- Universität Düsseldorf, Düsseldorf, Germany).

3. Sample Selection

3.a. Criteria of patient selection [6]:

Inclusion criteria:

Patients participating in this study were between 18-50 years old, able to tolerate necessary restorative procedures and having asymptomatic vital permanent teeth with dental caries including the inner 1/3 of dentin.

Exclusion criteria:

Medically compromised patients, pregnant women, patients showing allergy to any of the dental procedures, and uncooperative patients were excluded from the study. In addition, teeth with previous restorations, spontaneous pain, periapical lesions and positive response to percussion were also excluded from the current study.

3.b.Criteria for discontinuing or modifying intervention:

If patients showed any signs of severe postoperative pain, the restoration was removed, and endodontic treatment or extraction was performed.

4. Allocation & Randomization:

Randomization was conducted using a computer software (www.randomizer.org (Copyright© 1997-2021 by Geoffery C. Urbaniak and Scott Plous)). The number related to each intervention was written on cards placed inside sealed envelopes.

5. Blinding:

This trial was Single blinded, only the Outcome assessors were blinded, since the intervention approaches used for this study were obvious for the participants and principal investigator.

6. Sample grouping:

Patients were randomly divided into three equal groups based on the method of cavity disinfection:

1. **Application of Diode laser with 0.5 watt group (A_1):** patients with deep carious teeth received conservative caries management (selective caries removal to soft dentin) and then, a single application of Diode laser (EPIC X™, BIOLASE, USA) with 940 nm using 0.5 watt to the cavity was performed for 5 sec/mm^2 in a sweeping motion. Then, the teeth were restored with the final restoration.
2. **Application of Diode laser with 0.1 watt group (A_2):** patients with deep carious teeth received conservative caries management (selective caries removal to soft dentin) and then, a single application of Diode laser (EPIC X™, BIOLASE, USA) with 940 nm using 0.1 watt to the cavity was performed for 5 sec/mm^2 in a sweeping motion. Then, the teeth were restored with the final restoration.
3. **Chemical disinfection group (A_3):** patients with deep carious teeth received conservative caries management (selective caries removal to soft dentin) and then 2% Chlorhexidine (Consepsis®, Ultradent) was placed into the cavity for 20 seconds. Then, the teeth were restored with the final restoration.

II.1. General Operative procedures:

The procedures involved performing partial caries removal with cavity disinfection using either Diode laser with one of the two output powers or 2% Chlorhexidine (chemical disinfectant) for one tooth per patient. Patients were randomly selected into one of the three study groups.

Each patient signed an informed consent form after detailed and clear explanation of the study. Local anesthesia (Artinibsa 4% 1:100.000, Inibsa Dental S.L.U, Spain) was given and the tooth was isolated with a rubber dam (Sanctuary™ Dental Dam, Perak, Malaysia). The cavity was done with a #245 bur (Meisinger GmbH, Germany), using a high-speed handpiece *(Pana Air®, NSK, Japan)*.

All Caries was removed from the cavity walls, leaving at least 2 mm rim of peripheral sound tooth structure [17]. Only the first Superficial necrotic carious fragments were removed, leaving only soft dentin near the pulp [7].

2. Dentinal sample collection:

A Dentin specimen was then taken from the cavity using a sharp sterile excavator. Then, after cavity disinfection, another dentin sample was taken from the cavity and placed into Eppendorf tubes containing 0.5 ml of saline solution.

3. Intervention Application:

Group A_1: after taking the first dentinal sample, Diode laser (EPIC X™ , BIOLASE, USA) with 940nm using 0.5 watt was applied, with a 400μm noninitiated tip for 5 sec/mm^2 in a sweeping motion[15].

Group A_2: the cavity was disinfected using the same protocol as group A_1, only the output power was changed to 0.1 watt for 5 sec/mm^2 in a sweeping motion [18].

Group A_3: 2% Chlorhexidine (Consepsis®, Ultradent) was placed for 20 seconds, then air dried for 10 seconds for cavity disinfection [1].

4. Restorative Procedure:

Glass ionomer material (*Fuji II™ LC, GC Corporation, Tokyo, Japan*) following the manufacturer's instructions was first placed as a base, leaving a space 2mm distance for the final restoration. Then 35% phosphoric acid gel (Scotchbond™ Universal Etchant, 3M ESPE, Deutschland GMBH, Germany) was used for selective etching of enamel, followed by the universal adhesive (Single Bond Universal.3M ESPE, Germany), then light cured using LED light curing unit (Elipar ™ S10, 3M ESPE, USA) according to the manufacturer's instructions.

Resin composite (Filtek Z250, 3M ESPE, USA) was then applied in increments and each increment was light cured for 20 seconds, then the final increment was light cured for 40 seconds following the manufacturer's instructions. Occlusion was checked using an articulating paper (Blue Red Combo 0.0035''/89 μm, Crosstex® International. USA), and high spots were removed.

5. Microbiological Assessment:

The dentin specimens were cultured on three types of selective media; Blood agar for total viable count, Mitis salivurias agar for Streptococcus mutans, and Rogosa agar medium for Lactobacilli[1].

III. Statistical analysis:

Microbiological data were collected, tabulated, checked for normality, and statistically analyzed to evaluate the performance of different output powers of Diode laser and chemical disinfection. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests).

SPSS version 23.0 was used for data management and data analysis. Median and range when appropriate described quantitative data. Where data were non-normally distributed, the Wilcoxon Signed Ranks Test for paired comparisons and Kruskal-Wallis Test for more than two study groups comparisons. P-value was two tailed and considered significant at 0.05 level.

RESULTS

Microbiological Assessment:

1. Total Viable count of Blood Agar (TCBA):

Non-significant difference was found between the three studied groups regarding TCBA before and after application of the intervention. The p-value between the three studied groups before application of intervention was 0.143, while after was 0.241, both were statistically insignificant. The paired comparison of TCBA showed significant decrease in the three studied groups (Table 1).

2. Bacterial count of Mitis Salivurias (BCMS):

The current study results showed that there was non-significant difference between the three studied groups regarding BCMS before and after application of the intervention. The p-value between the three studied groups before application of intervention was 0.165, while after was 0.494, both were statistically insignificant. While the paired comparison of BCMS showed significant decrease in the three studied groups (Table 2).

Table (1) *Total Viable Count of Blood Agar before and after between study groups:*

		TCBA Before ($x10^3$)		TCBA After ($x10^3$)		P-value (paired comparison)
		Median	Range	Median	Range	
Group	A_1	128.70	77.60-220.70	1.00	0.30-1.70	**0.012***
	A_2	164.25	134.50-210.60	1.85	0.30-3.40	**0.017***
	A_3	197.40	128.90-260.10	1.70	0.60-3.10	**0.012***
P-value (between groups)		0.143		0.241		

**P-value is significant £0.05*

Table (2) *Bacterial Count of Mitis Salivurias before and after between study groups:*

		BCMS Before ($x10^3$)		BCMS After ($x10^3$)		P-value (paired comparison)
		Median	Range	Median	Range	
Group	A_1	141.60	69.20-200.30	0.95	0.20-2.10	**0.012***
	A_2	112.25	79.60-173.20	0.55	0.20-1.60	**0.012***
	A_3	80.75	56.90-190.70	0.50	0.20-2.10	**0.012***
P-value (between groups)		0.165		0.494		

**P-value is significant £0.05*

3. Bacterial count of Rogosa Agar (BCRA):

In the three study groups there was a non-significant difference in BCRA before and after application of the intervention. The P-value between the three studied groups before application of intervention was 0.358, while after was 0.385, both were statistically insignificant. Similarly, the paired comparison of BCRA showed significant decrease in the three studied groups (Table 3).

Table (3) *Bacterial count of Rogosa Agar before and after between study groups:*

		BCRA Before (x10^3)		BCRA After (x10^3)		P-value
		Median	Range	Median	Range	(paired comparison)
Group	A_1	61.50	49.90-82.40	0.80	0.20-1.60	**0.012***
	A_2	51.15	22.80-77.40	0.65	0.20-1.30	**0.012***
	A_3	59.20	24.50-72.40	0.50	0.20-0.80	**0.012***
P-value (between groups)		0.358		0.385		

**P-value is significant £0.05*

DISCUSSION

Invasive treatment strategies, such as one-step full caries removal.pose a considerable risk to the pulpal health of the tooth, including pulpal exposure. These invasive strategies assume that pulp vitality will deteriorate in the future, and that root canal treatment will be necessary. Although this strategy may be highly effective, it is more burdensome and costly on an individual and societal level [(5)].

To reduce unwanted pulpal exposure, two procedures can be used: stepwise excavation and partial caries removal [(19)]. Partial caries removal (PCR) is a one- or two-step procedure in which soft carious tissue towards the pulp is purposefully left in place and the cavity is permanently sealed, or a provisional restoration is employed for a pre-determined time interval before a final restoration is placed with no additional caries removal [(20)]. However, stepwise excavation (SW) is linked to higher expenses, and the risk of the patient failing to finish the final treatment step. These are substantial drawbacks for patients with inadequate financial resources or trouble accessing dental care [(17)].

Partial (selective) caries removal (PCR) is claimed to have a much greater success rate than stepwise excavation (SW), as it is said to result in fewer pulpal exposures. In addition, it was stated that when teeth were treated with Partial caries removal (PCR), they remained substantially more vital. When compared to single appointment Partial caries removal (PCR), stepwise excavations enhance the chance of pulp exposure. Also, many studies have stated that, sealing carious dentin tissues reduces the microbiological load in the infected dentin [(3)].

Still, it is of main concern to significantly decrease the remaining bacterial count present in infected dentin [21]. In the present study, disinfection of the remaining carious dentin was done using either Diode laser with one of the two output powers (0.5 watt or 0.1 watt) or 2% Chlorohexidine (chemical disinfectant).

Chlorohexidine is still claimed to be a gold standard antibacterial agent in dentistry because of its powerful antibacterial effect, which stems from the Chlorohexidine (CHX) molecule's ability to break the bacterial cell membrane, increasing permeability and causing cell lysis [22]. However, it is widely known that it may cause staining and discoloration to a tooth surface and might also have some cytotoxic effects against human fibroblasts through inhibition of protein synthesis [23].

Diode laser is gaining popularity in recent years and is considered to be a breakthrough in the field of dentistry as it enables dentists to perform many dental treatments in a faster and more efficient way[24]. It has a role in different procedures including soft tissue procedures mainly gingivectomy, frenectomy, gingival depigmentation, and many other surgeries. It is also used in teeth desensitization and whitening[25].

Regarding its antimicrobial effect, diode laser has its widespread application in periodontal pockets' disinfection and endodontic treatment owing to its ability to eliminate any remaining viable bacteria with great efficacy [26].

This antimicrobial impact is related to the temperature increase which causes the bacterial cells lysis; described as photothermal effect[27]. Moreover, it is worth mentioning that using diode laser with different parameters in various studies for direct pulp capping have shown high clinical outcomes [28, 29], as it is claimed that diode laser has the advantage of stimulating better repair and healing of pulpal tissues [30]. Therefore, cavity disinfection in the current study was done using diode laser with two different output powers (0.5) watt [15], and 0.1 watt [18], since they are safe parameters that have been previously used in cavity disinfection without causing any thermal damage to the pulp [15, 18], to compare their antimicrobial potential.

Microbiological assessment was performed in the current study, as streptococcus mutans and lactobacilli are the main bacteria responsible for producing dental caries in experimental animal and humans [31]. A culture-dependent approach is regarded as an effective approach for identifying viable bacteria [1].

Thus, the current study was carried out to assess the performance of Diode laser and 2% Chlorhexidine following selective caries removal.

The inclusion criteria of the current study were based on previously published studies eligibility criteria. Patients were considered eligible if they had teeth with primary carious lesions encompassing two-thirds of the dentin thickness but no evident pulp proximity on clinical and radiographic examination, this was supported by Sharma et al.[18] and Gamal et al.[32].

The method of randomization of the current study was performed through computed software (www.randomizer.org). In a study by Sharma et al.[18] and Matar et al.[22] subjects who met the inclusion criteria were assigned at random using a computer-generated list randomized into four equal groups. Using computer software as a method of randomization is accurate and not subjected to human error or bias.

The results of microbiological assessment showed that the bacterial count in the dentin samples decreased in the three tested groups after the application of the intervention. The bacterial count reduction using the two different parameters of diode laser have shown comparable results[1,33-35]. These results are attributed to the detrimental destruction to the bacterial cells by the thermal changes caused by diode laser.

Additionally, group A_3 (application of 2% Chlorhexidine disinfection) showed similar bacterial count reduction results. This was in accordance with previous studies carried out by Matar et al.[22] and Borges et al.[36] who stated that Chlorhexidine is an effective method in reducing the remaining bacteria in contaminated dentin. The disinfecting property of Chlorhexidine is dependent on the adsorption process of bacteria' cell wall, resulting in the release of internal components. Chlorhexidine has a bacteriostatic effect at low concentrations, resulting in the leaking of tiny molecular weight compounds from microorganisms. Chlorhexidine has a bactericidal action at higher concentrations due to cytoplasmic precipitation and coagulation, which was presumably produced by protein cross-linkage. Additionally, the cationic characteristics of chlorhexidine allow it to infiltrate more deeply into dentin and extend its sustained antibacterial potential [37].

Contrary to our findings, the results and conclusions of previous studies conducted by Uday Mohan et al.[1] and Taha et al.[33] revealed that the reductions in bacterial counts with cavity disinfectants with 2% Chlorhexidine showed the highest reductions of the bacterial content, followed by Diode Laser, such results may be due to the different laser power used by those studies.

The findings of the current study could still favor the usage of diode laser in management of deep carious lesion, because of its promising results in disinfection, that might lead to better clinical outcomes. However, further clinical studies are recommended with long term follow up periods and larger sample size to achieve more conclusive results.

CONCLUSIONS

Within the limitations of the current study, the following can be concluded:

1. Diode laser can show promising results in terms of cavity disinfection and management of deep caries lesions.
2. Chlorhexidine is as effective as diode laser in cavity disinfection.

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