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Effect of Diode Laser Versus Chlorhexidine on Bacterial Reduction in Pulpectomy of Primary Molars: An In-vitro-Study

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Aim: This study was based on evaluating the bactericidal ability of diode laser (980 nm) in root canals of primary teeth infected with Enterococcus faecalis.
Materials and methods: Forty-eight primary molar teeth were collected and divided into four groups (n = 12).
Group I: Chlorhexidine (CHX), Group II: 980 nm diode laser, Group III: CHX + diode laser, Group IV: Saline (positive control). Distal canals were separated, cleaned and shaped using manual K-files, sterilized then infected with E.feacalis. Samples from the infected root canals were obtained and cultured on agar. The numbers of CFU were counted after each intervention at 24 h and at 7 days. Data were analyzed by Post hoc pairwise comparisons.
Results: The highest percentage of bacterial reduction in 24 h and 7 days was found in laser/CHX group and CHX group with no significant difference between these two groups at both time intervals ($p>0.05$). Followed by laser group while the saline group had the lowest value after 24 h and 7 days
Conclusion: Laser can be used as successful approach solely or in conjunction with biocompatible irrigating materials in pediatric patients for disinfecting root canal

Keywords: Laser, Pulpectomy, Enterococcus faecalis, Disinfection

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Introduction

Pulpectomy treatment in primary teeth principally aims to eliminate the microbial load in the root canal system. [1] to sustain the presence of these teeth until exfoliation time. Pulpectomy in children's primary molars is constantly facing several obstacles, such as primary molars root curvature, root canal complexity, ramifications and physiological resorption. Nevertheless, children's tolerance, co-operation, ability to keep mouth open and limitations of accessibility make pulpectomy procedures even harder. It is yet difficult to achieve complete pulp extirpation and disinfection of all root canals under these circumstances. [2]

Root canal systems of primary teeth tend to have a poly-microbial nature of microbes similar to those of permanent teeth. Anaerobic bacteria have predominantly been identified from samples of either necrotic pulp or periapical lesions in deciduous teeth.[3] The Enterococcus faecalis (E.faecalis) predominantly occupied the root canal systems in almost 30 % of the specimens. Other bacteria were also isolated but in less dominance like the: Escherichia coli, Staphylococcus aureus, α-hemolytic Streptococci and Proteus mirabilis. [4] Persistent peri-radicular lesion is a common finding following some root canal therapies. Such resistant infections are most like associated by the presence of the E.faecalis as this organism has an ability to thrive and is mainly responsible for multiple root canal therapy failures. [5]

Mechanical instrumentation of root canals of primary teeth is an inevitable step in the direction to remove pathogens from the root canals. Yet, this procedure still needs adjunctive cleaning protocols to ensure the complete cleaning of the walls of the root canal system. In the event of insufficient cleaning of the canal walls, a large number of microorganisms and associated necrotic pulp tissue might still be stuck to the walls, dentinal tubules and unreachable accessory canals. [6] Therefore, bacterial reduction by employing intracanal biocompatible medicaments/ irrigants is essential. Nevertheless, the problem faced by pediatric dentists is the selection of the most suitable medicaments/ irrigants during pulp canal treatments, owing to the possible harm to the successor tooth, [2] accidental ingestion or even the passage of such medicaments/ irrigants beyond the physiological apex.

Although Sodium hypochlorite (NaOCl) is considered the golden standard in disinfection of root canals. Yet, its tissue toxicity, risk of ingestion, unpleasant odor and preferable rubber damn placement refrains its use in children. [7,8]

Chlorhexidine gluconate (CHX) is one of the fundamental irrigants in root canal disinfection protocols, particularly in primary teeth. CHX is characterized by its stable nature, extended- action and broad-spectrum antimicrobial properties. One of the major advantages of CHX is its antibacterial substantivity, which can last for up to 3 months, minimizing bacterial proliferation and exerting an extended antibacterial effect within the root canal system of primary teeth. Moreover, its biocompatibility especially in periapical tissues makes it a valuable and safe irrigant in primary teeth. [9] Yet, research has found that CHX needs to be used in conjunction with other irrigants to ensure a comprehensive cleaning and disinfection to achieve a successful endodontic treatment by ensuring the dissolution of necrotic pulp tissue. [10,11]

Research has shown that dental lasers have a promising application in the field of root canal therapy providing an efficient cleaning process and therapy outcome. Various types of lasers like the diode lasers, Erbium family lasers and even Nd:YAG can aid in a successful pulp therapy by removing smear layer and disinfecting the canal by its killing action to the bacteria. [12]

Laser disinfection of primary root canals is dependent on several parameters involving the wavelength, power,

irradiation duration and mode of emission. Studies have highlighted the importance of these parameters on the efficacy of canals disinfection. [13]

The photo-thermal effect produced by diode lasers can help in bacterial load reduction by disrupting the bacterial cell wall and destroying its cell membrane. On the other hand, the photo-acoustic effect produced by Erbium lasers help in exposing the dentinal tubules to irrigants by the removal of the obstructing smear layer. [14] Though lasers have shown ability to disinfect root canals. Yet, they cannot deliberately replace conventional disinfection methods. Thus, further research is required to explore the full potential of lasers in disinfecting root canals especially in primary molars, to optimize disinfection protocols and ensure successful pulpectomy. [15]

In the current study, the difference in the bacterial reduction of infected primary root canals with E.feacalis after irrigation with CHX, irradiation with diode laser (980 nm, Photon Plus, Zolar Tech & Mfg Co. Inc, Ontario, Canada), or in combination was evaluated. No difference in the outcome among the different tested disinfecting methods was the null hypothesis.

Materials and methods

Ethical Approval

This study was reviewed and accepted by the Ethical Committee Board of Faculty of Dentistry, Ain Shams University with reference number FDASU-REC IR121912.

Sample size

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there's no difference in bacterial reduction measured in tested groups. By adopting alpha (α) and beta (β) levels of (0.05), (i.e., power=95%), and effect size (f) of (13.41) calculated based on the results of a previous study. [12]; the minimum total required sample size (n) was found to be (6) samples. The sample size was increased to (12) to account for possible procedural errors during testing. Sample size calculation was performed using R statistical analysis software version 4.4.0 for Windows. [16]

Sample preparation

Freshly extracted retained primary mandibular secondary molars (n=48) were cleaned and rinsed under water and kept in saline at room temperature. Specimens were selected to follow the inclusion criteria. Inclusion criteria included: 1) Patent distal canal free from previous treatment and fully formed apex; 2) Distal canal with 10-20° curvature according to Schneider method [17]; 3) no internal or external root resorption; 4) Type I canals in the distal root. Teeth that didn't fit to the inclusion criteria were discarded.

Selected teeth were de-coronated and the distal roots were separated and adjusted to a length of 7mm for standardization purposes. Patency for all distal roots was primarily checked using K-file size # 10. A barbed broach was used to remove any remnants of pulp tissue. Cleaning and shaping was conducted to all distal roots using manual stainless-steel K-files through crown-down technique up to size #35 (Dentsply Maillefer Ballaigues, Switzerland) by a single operator. Roots were then irrigated using 2ml of 5.25 % NaOCl solution delivered using a plastic syringe of a 27-gauge needle (Endo Eze; Ultradent Products Inc, South Jordan, UT). Patency was rechecked after each file to avoid canal blockage by debris.

Samples were then divided into four groups randomly according to the assigned treatment modality. Sterilization of all roots was conducted in autoclave at 121 °C for 15 minutes. The external surface of the roots were coated with epoxy varnish to avoid bacterial contamination, apices were also sealed by paraffin wax. The four treatment groups involved; **Group I:** CHX, **Group II:** Diode laser, **Group III:** CHX + diode laser, **Group IV:** Saline (positive control). Roots were then placed in a sterile test tube with 5 ml sterile tryptone soy broth (TSB)

(Oxoid LTD, England). All Roots were verified for sterility after 7 days.

Contamination of distal root canals with E. faecalis and culture

E. feacalis (ATCC4083) was inoculated on brain heart infusion (BHI, Oxoid LTD, England) and incubated at 37°C for 24 h in a CO_2 incubator (Sanyo MCO-18AIC, Japan). A 1 McFarland standard suspension was prepared on BHI broth and then diluted 30-fold to obtain an initial bacterial suspension of 1.5 x 10^8 colony-forming units (CFU) per milliliter. Sterilized samples were inoculated with the initial bacterial suspension under a laminar flow cabinet using a sterile pipette.

K-type files #15 were used to transport the bacterial suspension to the full length of the canal. [18] Samples were then incubated for 4 weeks at 37 °C, BHI medium was refreshed every week. Samples were then removed from the tubes and placed in pre-fitted acrylic blocks. A size #15 K-file (Dentsply Maillefer) was used to circumferentially instrument the canals for 10 s till the full working length. A sterile size # 20 paper points (Dentsply Maillefer) were inserted in each sample root canal for 10 s to collect the bacterial sample. This step was done for each sample using three paper points. The sampled paper points were transferred to an Eppendorf tube containing 500 lL of BHI broth and vortexed for 30 s for serial dilutions. Baseline quantitative bacterial assessment (B) was done by inoculating each dilution on BHI agar medium plates incubated at 37 °C for 48 h. Baseline CFUs were further assessed.

Root canal disinfection

All infected root canals were placed were assigned to each group:

Group I: CHX, **Group II:** Diode laser, **Group III:** CHX + diode laser, **Group IV:** Saline (positive control).

Group I: CHX group, distal canals were irrigated with 2ml of 2% CHX (Dentochlor, Ammdent, 2% Chlorhexidine solution) for 60s using a 27-gauge needle syringe for 60s. [19]

Group II: Diode laser group, distal canals were irradiated with a 980 nm diode laser at an output power of 1.5 in continuous mode, non-initiated tip, non-contact mode (980 nm, Photon Plus, Zolar Tech & Mfg Co. Inc, Ontario, Canada). An optical fiber 200 µm in diameter was inserted into the root canal 1 mm short of the working length. The irradiation was conducted up to four times in circular motion from down to up at 10-s intervals/time. [20]

Group III: Distal root canals were irradiated using diode laser as in group II and were conducted four times. During each irradiation, the canals were irrigated with 2ml of 2% CHX using a sterile syringe. [21]

Group IV: Saline (Positive control group), distal canals were irrigated with 2 ml saline using a 27-gauge needle syringe for 60s.

Microbiological assessment:

Each distal canal was sampled as described before, to have the post-intervention microbiological samples at 24 h and at 7 days post intervention. I_1 and I_2 samples were vortexed for 30s and inoculated on bile euscalin agar for E.faecalis at 37 °C for 24 h. Bacterial colonies were counted and results were expressed as the number of CFU per milliliter.

Statistical Analysis

Numerical data were presented as mean, standard deviation (SD). They were tested for normality by viewing distribution and using Shapiro-Wilk's. They were found to be non-parametric and were analyzed using Kruskal-Wallis's test, followed by Dunn's post hoc test for intergroup comparisons. P-values were corrected for multiple comparisons using the False Discovery Rate (FDR) method. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.4.0 for Windows. [16]

Results

The highest percentages of bacterial reduction in 24 h and 7 days was found in

Laser/CHX group (after 24 h: 100.00±0.01 and 7 days: 100.00±0.00) and CHX group (after 24 h: 99.04±1.38 / 7 days: 99.76±0.34) with no statistically significant difference between these two groups at both time intervals (p>0.05). Followed by diode laser group (after 24 h: 83.01±14.82/ 7 days: 93.19±6.70) while the control group had the lowest bacterial reduction value (after 24 h: 50.55±5.58 / 7 days: 38.33±6.39). Post hoc pairwise comparisons showed a statistically significant difference among the four experimental groups (<0.001*) Tables (1). Although, bacterial reduction values increased after 7 days for the laser group, the difference was also not statistically significant. Summary statistics for bacterial reduction data are presented in Figures (1) and (2).

Table 1: Percentage change of bacterial count (Mean±SD)

Time Interval	*Mean ± SD*				*p-value*
	Saline	*Chlorhexidine*	*Laser*	*Laser and chlorhexidine*	
24 hours	50.55±5.58 ^{C}	99.04±1.38 ^{AB}	83.01±14.82 ^{B}	100.00±0.01 ^{A}	**<0.001***
7 days	38.33±6.39 ^{C}	99.76±0.34 ^{AB}	93.19±6.70 ^{B}	100.00±0.00 ^{A}	**<0.001***
p-value	**0.036***	**0.584**	**0.295**	1	

Values with different superscripts within the same horizontal row are significantly different, *significant (p<0.05)

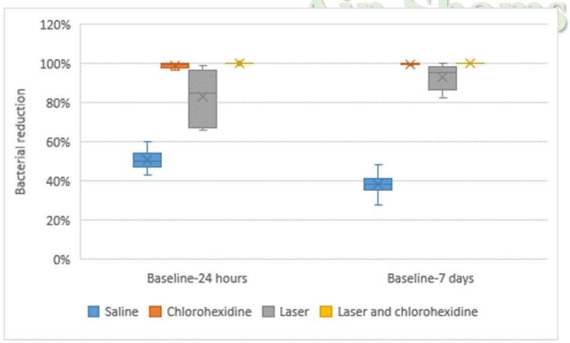

Figure 1: Box plot for bacterial reduction (A).

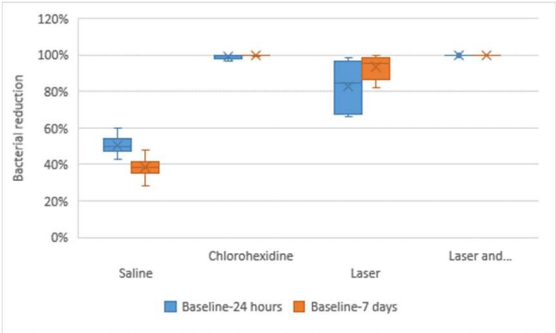

Figure 2: Box plot for bacterial reduction (B).

Discussion

Root canal therapy for primary teeth involves cleaning and shaping the canals to remove microorganisms. Proper disinfection is an essential step for long-term success, yet it can be challenging due to complex anatomy and bacterial load within these canals.

This study aimed to assess the effectiveness of CHX, laser irradiation and the combination of both techniques since the use of NaOCl in pediatric dentistry is not highly recommended. On the contrast to adults, The roots of primary teeth is in an undergoing phase of physiological resorption process which can start as early as 4 years of age and that makes it hard to create an apical stop when conducting root canal therapy. The inability to create an apical stop makes it easier for irrigating solutions such as NaOCl to pass the apex and thus would be irritating to the peridontium. Accordingly, CHX was selected in this study as it is considered a biocompatible material, less irritating to tissues. Yet, it has some limitations and for this sake, lasers were selected in this study to be used alone or as an adjunctive to CHX to test their efficiency in cleansing the root canals of primary teeth against the most dominant organism E. faecalis. Where E. faecalis prevalence rate was reported to be from 22 to 77% of the cases with infected root canals. [22]

Use of Lasers as an adjunctive tool to cleanse infected root canal has been highly recommended lately. This is attributed to the ability of the high intensity light beam to reach deeper layers in

dentinal tubules killing the bacteria at deeper levels that couldn't be reached by the irrigants alone. The reported penetration depth is around 500 um in dentin. Moreover, the complex root canal system of primary teeth has made the regular cleaning and shaping protocols insufficient to reach the lateral ramifications. This complex anatomy has made some infections resistant to regular cleaning protocols. The use of diode lasers in such cases can help eliminate such resistant bacteria from unreachable spaces within the root system. [23]

Based on the current research results provided and by reviewing previous research, there are several studies that compared the effectiveness of laser irradiation to other irrigants for root canal disinfection in primary teeth. The results of our study stated that there was a statistically significant difference among different tested groups. The highest bacterial reduction was found in the CHX in conjunction with laser group, followed by the CHX with no statistically significant between both groups followed by the laser group.

Our results came in agreement with Walia 2019, [8] who compared the efficacy of 2% CHX, 1% sodium hypochlorite, and laser irradiation in reducing root canal infection in primary teeth. The results showed that all three methods succeeded in reducing the root canal infection. Yet, there were no reported significant differences between tested groups.

Moreover, results of this study came in alignment with Botu 2023 [24] and Ashofteh 2013, [25] where diode laser irradiation had similar root canal disinfection efficacy compared to 2% CHX in primary canals. It was agreed that diode lasers could be used as an alternative to chemical disinfection methods in primary teeth. Also current results were further affirmed by another study, evaluating the effect of two laser systems, one of them was a 940 nm diode laser - on root canal disinfection. The results indicated that laser systems were effective in reducing bacterial counts in root canals, with no significant differences between the two laser types used. [12]

Similar results came in agreement with the present study results were diode 810nm showed effectiveness in elimination of E.faecalis up to 500um in dentin. [26,27,28] Various powers for diode lasers 809nm (1/5,3,4/5W) were tested in a study using a 200um diameter tip for 60 sec, results confirmed that the combination of laser with irrigating solutions provided the optimum bacterial reduction. [29]

Gutknecht 2000, [27] results came in accordance to this study result where a 980-diode laser was used to eliminate bacteria up to 500um in dentin.

Similarly, Santos 2013,[30] concluded that high power diode laser when associated with CHX gluconate 2% improves that bactericidal effect of the irrigating solution in infected root canals.

With all the promising results with diode lasers, yet, the mal-use of dental lasers with improper adjustments to the parameters can cause damages to periapical area and vital structures in close proximity to the tooth i.e; inferior alveolar nerve, mental foramen or even maxillary sinus. This damage can arise from the temperature rise caused by the diode lasers photothermal effect. [31]

Conclusion

Diode laser in conjunction with CHX provides the best effect in root canal disinfection in primary teeth especially with resistant infections. Diode lasers shall be used within the proper parameters to ensure disinfection with minimal hazards. CHX can also be used alone in disinfecting primary root canals whenever diode lasers are not available.

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Data availability: The data generated during the current study are available on reasonable request from the corresponding author.

Competing interests: The authors have

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