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ANTIBACTERIAL EFFECT OF CHITOSAN AND LOW-LEVEL DIODE LASER AGAINST ENTEROCOCCUS FAECALIS IN INFECTED ROOT CANALS IN PRIMARY MOLARS

Waleed O. Amin Kadour[1*], Mohamed Abu El-Maaty Mohamed Abu El-Hasan[2], Ibrahim Farouk Barakat[3]

ABSTRACT

Objectives: Infected root canals contain resistant bacteria with high resistance to antibacterial solutions such as Enterococcus faecalis (E. faecalis). Therefore, this study was aimed to evaluate and compare the antibacterial effect of the chitosan (CH) and Diode laser on an infected root canal. **Subjects and Methods:** Four disinfectant systems were used in the present study; 2.5% sodium hypochlorite (NaOCl) "control group", 0.2% chitosan solution, low-level diode laser (1.5-watt), and chitosan/diode laser in combination. This study involved a total of sixty-four carious primary molar teeth indicated for pulpectomy in children aged between 4-7 years. **Results:** All disinfectant systems showed a statistically significant effect against E. faecalis. There was a statistically significant difference between the four tested systems, while among the groups, there is no statistically difference between NaOCl and diode laser, and between diode laser and combination of chitosan/diode laser. **Conclusion:** The use of chitosan and laser alone or in combination has a significant effect on the reduction of E. faecalis count. However, the use of diode laser and combination of chitosan/diode laser as disinfectant systems showed a statistically comparable result to NaOCl.

KEYWORDS: Antibacterial, Chitosan, Enterococcus faecalis, Infected Root Canals, Laser, Primary Molars

INTRODUCTION

During root canal treatment as in the case of pulpectomy of primary teeth, the removal of the contaminated tissues from the infected root canal using mechanical instrumentation is considered the main goal [1,2]. However, mechanical instrumentation cannot completely eradicate the present bacteria and their toxins products[3]. This incomplete bacterial eradication usually occurs because of the presence of accessory canals and other anatomical variations[4].

Therefore, the use of disinfectant agents or systems that poses antibacterial activity was strongly recommended as an effective method that could help the mechanical instrumentation in bacterial eradication [2,5]. Although, the efficacy of these antibacterial agents has controversial results as well as they could have some adverse effects [6].

1. Assistant lecturer, Pedodontics and Oral health Department, Faculty of Dental Medicine for Boys, Al-Azhar University, Egypt.
2. Professor, Pedodontics and Oral Health Department, Faculty of Dental Medicine for Boys, Al-Azhar University, Egypt.
3. Assistant Professor, Pedodontics and Oral Health Department, Faculty of Dental Medicine for Boys, Al-Azhar University, Egypt

• ***Corresponding author*:** dr.waled.omar@gmail.com

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For example, NaOCl is the commonest irrigant solution which widely used during root canal treatment because of its capacity to dissolve the residual necrotic tissues as well as its antibacterial activity [7]. However, NaOCl in full concentration has some disadvantages including cytotoxicity, bad test as well as an offensive smell [8]. Additionally, NaOCl cannot kill all bacterial strains and has limited penetrating depth in dentinal tubules of 130 μm [3,8]. Furthermore, the Sodium hypochlorite with lower concentrations also has been used such as 0.5% or 1% with significant antibacterial effect near or equal to the full concentration. [9].

Therefore, numerous novel products are recently used as root canal irrigant with different chemical properties [10]. These products were compared in previous studies regarding their antimicrobial effect as well as biocompatibility to state the most appropriate antibacterial agent that can use as a root canal irrigant [11].

Recently, chitosan as a natural cationic polysaccharide extracted from crustaceans has great attention because of its excellent biocompatibility [12]. Chitosan has a considerable antibacterial effect through increasing the permeability of the bacterial cell wall via the bonding of its cationic group with the negatively charged cell wall [13].

The diode laser has been established in dental practice because of its acceptable temperature rise as well as its bactericidal effect, and its relative economic price [14]. Moreover, it has been concerned recently in root canal treatment because of its bactericidal effect as well as its higher ability to penetrates the dentinal tubules to more than 1 mm of its thickness [14].

Therefore, this study was directed to clinically evaluate and compare the antibacterial effect of chitosan, and diode laser alone or in combination with traditional root canal irrigant (NaOCl) in primary molars root canals infected with E. faecalis organisms.

SUBJECT AND METHODS

This experimental prospective controlled clinical study was carried out on children aged from 4-7 years after approval of Ethical Committee, Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo) with approval reference No (84/96). The involved children were selected from outpatients of the Pedodontics and Oral Health Department, Faculty of Dental Medicine, Al-Azhar University (Cairo, Boys). This study involved sixty-four carious primary molar teeth indicated for pulpectomy which randomly divided into four equal groups (n=16) according to the used disinfectant system; group I; 2.5% sodium hypochlorite as the control group, group II; 0.2% chitosan solution, group III; diode laser with a power of 1.5 watts, and group IV; chitosan/diode laser in combination. The sample size was determined for this study based on the previous results of Kapadia et al, [13].

Subject selection:

Verbal and written consent was obtained from the parents of each enrolled child before starting this study. Inclusion of children in this study was based on the absence of any systemic diseases with no history of antibiotic or anti-inflammatory treatment for the last 2 weeks before starting this study, presence of carious primary molars that indicated for pulpotomy, no root resorption more than one-third of its radiographic length, as well as the presence of adequate tooth structure to be restored with stainless steel crown [6,12].

Preparation of chitosan solution:

Preparation of 0.2% chitosan irrigant solutions was performed using 0.2 grams of chitosan powder (Sigma Co., Egypt), diluted in 100 ml of 1% acetic acid, and the mixture was stirred for 2h using a magnetic stirrer until completely dissolved. The chitosan irrigant solution was adjusted at a pH of 3.5 with sodium hydroxide using a digital pH meter. The prepared solution was kept in the refrigerator and used within two weeks after preparation [12].

Operative procedures:

Pulpectomy procedures:

To confirm the diagnosis of the intended tooth a periapical radiograph was achieved before starting the pulpectomy treatment. Then, the operative site was locally anesthetized and isolated with a rubber dam. A sterile excavator and handpiece were used to remove any existent caries until identifying the exposure site. Thereafter, deroofing and access cavity preparation was carried out with a sterile fissure bur. Then, the coronal pulp tissue was removed with an excavator and the pulp chamber was debride with saline. To complete the root canals preparation; the rotary endodontic files system was used until size 30. During the mechanical instrumentation procedure, the root canals were irrigated with saline using a sterile disposable 26-gauge needle. After that, the root canals were dried with sterile paper points size 30, and sterilized cotton pellets were positioned in the root canal orifices [15].

Root canals disinfected protocol:

After pulpectomy procedures, the root canals were carefully disinfected with the suitable disinfected protocol according to the pre-allocated sample grouping in this study. In group, I and group II; NaOCl and chitosan irrigant solutions were introduced into the root canals until 2 mm of the working length without excessive pressure [12].

Before laser use, the child and operator wear protective eyeglass. In group III; the optical fiber of continous diode laser (lasotronic lases Co. Netherlands) with 200µm diameter and power of 1.5 watts was introduced into the root canal with a length shorter of 1 mm than the working length. The optic fiber tip was moved in circular slow movements for 15 seconds from direction started from the apical to the coronal part of the root canal (recommended by the manufacturer) and this procedure was repeated four times [16]. While, for group IV; the root canals were disinfected with 0.2% Chitosan irrigant solution with the same protocol done in group II, followed by diode laser disinfection with the same protocol in group III. Finally, after disinfection, the root canals in all groups were dried with sterile paper points size 30.

Restoration procedures:

After disinfection and microbiological sampling, a thin mix of zinc oxide and eugenol cement was prepared and inserted into each root canal with an endodontic plugger [17]. The pulp chamber then was filled with a layer of glass ionomer cement and the remaining part was filled with resin-modified glass ionomer as the final restoration. Then each tooth was restored chrome steel crown [12].

Microbiological sampling:

After access opening the first microbiological sample (S1) was collected from each root canal by sterile wet paper point of a compatible size. Each paper point was introduced into the root canals to a full working length for 1 min. After ending the different disinfection protocols a second microbiological sample (S2) was taken immediately in a similar way as the first microbiological samples (S1) [18]. Then each collected paper point in first or second microbiological sampling was placed immediately in labeled tighten screw-capped vial containing 2ml peptone liquid media as transfer media. Peptone liquid medium is a diluent and non-selective pre-enrichment used to keep the viability of the bacteria.

Then, all collected microbiological samples were transported, under complete aseptic condition, as soon as possible to the microbiological lab for culture procedure on the selective *Enterococci* agar media. After incubation for 7 days at 37°C, the colonies were identified, counted, and expressed as the total colony-forming units per ml (CFU/ml) [18].

All collected data were then tabulated and statistically analyzed using **a** one-way ANOVA test

to compare between groups and Paired *t-test* and were used to compare in-between the same group means for quantitative data with normal distribution. The results were considered statistically significant at $p<0.05$.

RESULTS

The paired t-test results revealed a statistically significant reduction in E. faecalis counting after the use of different disinfectant protocols ($p< 0.0001$) (Table 1). The results of counting *E. faecalis* in the four studied groups at the baseline showed a non-statistically significant difference ($p=0.870$). However, after the use of different disinfectant protocols, there was a statistically significant difference between groups ($p<0.00001$) by using One-way ANOVA test and the pairwise comparison test (post-Hock) showed a statistically significant difference in-between (group I and II), (group II and III), and (group II and IV) with the level of significance of $p=0.00000$. While, no statistically significant difference in-between (group I and III), (group I and IV), and (group III and IV) with the level of significance of $p=0.32405$, $p=0.99908$, and $p=0.39743$ respectively (Table 2).

TABLE 1: Comparison of E. faecalis count (CFU/ml) before and after different disinfection protocols.

Variable	Base-line	After Disinfection	*p*-value
	Mean± SD	Mean± SD	
NaOCl	2810.63±613.36	1029.88±93.12	<0.0001*
Chitosan	2765.00±442.79	1646.13±279.31	<0.0001*
Laser	2893.31±513.30	1143.00±139.84	<0.0001*
Chitosan/laser	2917.56±732.01	1038.88±180.78	<0.0001*

**; significant at p < 0.05.*

Table 2: Descriptive statistics of E. faecalis count (CFU/ml) along with the study.

Variable	Base-line	*p*-value	After disinfection	*p*-value
	Mean± SD		Mean± SD	
NaOCl	2810.63 ±613.36	0.870	1029.88 ±93.12	<0.00001*
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Laser	2893.31 ±513.30		1143.00 ±139.84	
Laser/Chitosan	2917.56 ±732.01		1038.88 ±180.78	

**; significant at p < 0.05.*

; non-significant at p >0.05.

DISCUSSION

The root canal treatment in children is called pulpectomy which is a clinical procedure to remove the necrotic or irreversibly inflamed pulp tissues[19]. The necrotic and/or irreversibly inflamed pulp tissue should be removed to prevent the development of any preapical pathosis that could affect the permanent tooth germ [20]. During the procedures of pulpectomy, the root canal is shaped with hand/rotary instruments and then debride with antimicrobial agents [21].

In the present study, NaOCl was chosen as the control group because it is the gold standard irrigant solution due to its higher antibacterial capacity [22]. In this study, the 2.5% concentration of NaOCl was chosen to avid its high toxicity [23]. Also, chitosan was chosen as an irrigant solution because it has strong bactericidal activity and is not toxic for mammals [24]. However, diode laser was chosen as a disinfectant system because laser achieves better disinfection of root canal system by deep penetrating and cleaning complex endodontic system [18]. Moreover, in the current study, a combination of chitosan and diode laser was chosen as a new disinfectant technology

as this combination can strong synergistic effect and improve its antibacterial efficacy [25].

In this study, E. faecalis was chosen as the test microorganism because it is the prime cause for root canal therapy failures due to its high survival ability in a variety and adverse environments and its ability to withstand the antibacterial effect of root canal medicaments [18,26]. This study detected a high number of E. faecalis in the infected root canals at the baseline with no statistically significant differences between the tested groups, so this referred to standardization for bacterial counts in all groups [18].

The finding of this work revealed that the use of NaOCl as an irrigant solution can significantly decrease the colony count of E. faecalis. This may be due to the formation of chloramines from the dissociation of NaOCl which interferes with cellular metabolism [7].

Also, it was found that the use of chitosan can significantly decrease the colony count of E. faecalis. This may be because of the alkalinity and hydrophilicity of chitosan as well as its ability to change the membrane's permeability of the bacteria[25]. While the significant reduction in bacterial colony count of E. faecalis after the use of laser. This may be because the Laser light can kill the microorganisms by generated energy [18]. Moreover, the finding of this study showed a significant reduction in the bacterial colony count of E. faecalis after the use of chitosan/laser combination. This may be because that laser can excite the chitosan particles to cause photo-thermal lysis as well as the use of this combination has a strong synergistic effect and improve its antibacterial efficacy [25].

CONCLUSION

From the finding of this study, it could be concluded that the use of chitosan, laser, or a combination of both was effective in reducing the E. faecalis count in pulpectomized teeth.

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