EVALUATION OF ANTIBACTERIAL ACTIVITY OF CHITOSAN AND DIODE LASER IN CONTACT WITH STAPHYLOCOCCUS AUREUS IN PRIMARY MOLARS INFECTED ROOT CANALS

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ABSTRACT:

Objectives: Staphylococcus aureus (S. aureus) is a resistant bacterium which usually inherent and frequently isolated from root canal infection. Hence, this work was directed to evaluate the efficacy of chitosan (CH) and Diode laser against S. aureus in infected root canals of primary molars.

Materials and Methods: This clinical study was conducted on a group of children aged between 4-7 years with decayed primary molars indicated for pulpotomy. Primary molars are categorized into four groups according to the disinfection protocol: 2.5% sodium hypochlorite (NaOCl) “control group”, 0.2% chitosan solution, diode laser (low-level of 1.5-watt), and combination of chitosan/diode laser.

Results: All tested disinfectant protocols significantly reduce the S. aureus count. Also, the statistical comparison of tested disinfectant protocols exhibited a significant difference regarding S. aureus count. While in between the groups, there is no statistical difference in between NaOCl and diode laser, and in-between chitosan and diode laser.

Conclusion: The chitosan/diode laser combination as disinfectant protocol revealed the higher significant effect on the reduction of S. aureus count in comparison to other protocols. However, the use of chitosan or laser alone has a significant effect on the reduction of S. aureus count.

Keywords: Antibacterial, Chitosan, Infected Root Canals, Laser, Primary Molars, Staphylococcus aureus.

I. INTRODUCTION

Pulp therapy is the only treatment option for the irreversibly inflamed, infected root canal because the necrotic pulp has a defense mechanism or antimicrobial activity as well as it cannot initiate an immune response¹. Pulpectomy in primary teeth is the synonym to the endodontic treatment in permanent dentition². A pulpectomy is defined in pediatric dentistry as the clinical procedure to eliminate the infected or irreversibly inflamed pulp tissues to prevent the incidence of any preapical pathosis that could affect the tooth germs of permanent dentition¹,³. One of the most important procedures during pulpectomy is root canal debridement via using different disinfectant protocols⁴.

The microbial organisms in the infected root canal play a significant role in the initiation of the process of inflammation and hence necrosis pulp tissues and finally the formation of preapical pathosis⁵. S. aureus is one of the commonest microbial organisms that are present in the infected root canal⁶. It is gram-positive bacteria that have high resistance to the chemical agents during pulp therapy⁷. Additionally, S. aureus plays an important role in primary and recurrent endodontic infections⁷,⁸.
Hence, the entire eradication of these pathogenic microorganisms from the infected root canals is one of the main objectives of successful endodontic treatment. However, the entire eradication is difficult, therefore, bacterial load reduction was the alternative goal that can achieve by various disinfection procedures such as mechanical instrumentation, and the use of antimicrobial agents or drugs.

Sodium hypochlorite is the universal irrigant agent due to its extensive antibacterial activity and its ability to dissolve the necrotic pulp tissue. The antibacterial activity of NaOCl increases with the increase of its concentration, although its cytotoxicity also increases. Chitosan is a new natural and biocompatible material with acceptable antimicrobial activity. Chitosan is a natural polysaccharide that has a positive charge which able to change the cell wall permeability of the targeted bacteria.

The laser can consider as an alternative tool for root canal disinfection through penetrating and decontaminating the infected and complicated root canal system via its photo-thermal properties. This is because the laser can generate heat and its light able to deeply penetrate the dentinal structure into a depth of more than 1000 micrometers.

Therefore, the present study was aimed to clinically evaluate the antibacterial effect of chitosan, and diode laser alone or in combination and compare it with NaOCl against S. aureus microorganism in the primary molars with an infected root canal.

II. SUBJECT AND METHODS:

This study was designed as an experimental prospective controlled clinical study. After ethical approval was obtained from Ethical Committee, Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo) with approval reference No (84/96), the present study was conducted on a group of children aged between 4-7 years. The selection of the enrolled children was from outpatients of Pedodontics and Oral Health Department, Faculty of Dental Medicine, Al-Azhar University (Cairo, Boys). The present study included a total of 64 carious primary molars registered for pulpectomy procedures. The involved teeth were divided randomly into 4 equal groups (n=16) based on the used disinfectant protocol; group I: 2.5% NaOCl “control group”, group II: 0.2% CH solution, group III: diode laser with low power of 1.5 watts, and group IV: a combination of chitosan/diode laser. A sample size of 16 teeth was recorded for this study depend on the results of previous by Kapadia et al.

Subject selection:
Children enrolled in the present study haveno history of any systemic diseases as well as no history of drug medication with anti-inflammatory or antibiotic treatment before starting this study for the last 2 weeks. The involved teeth were indicated for pulpotomy, with no apical root resorption greater than one-third of its length. Also, involved teeth should have adequate coronal structure to be reestablished with stainless steel (SS) crown. Before starting this study written consent was collected from parents/caregivers of each included child.

Chitosan solution preparation:
A concentration of 0.2% chitosan solutions was prepared using 0.2 grams of chitosan powder (Sigma Co., Egypt). Chitosan powder was diluted in 100 ml of 1% acetic acid and mixed vigorously in a magnetic stirrer for 2 hours until completely dissolved. The pH of the chitosan solution was adjusted with NaOH with the aid of a digital pH meter at 3.5. Then, the prepared chitosan solution was kept in the refrigerator and used within two weeks after preparation.

III. OPERATIVE PROCEDURES:

Pulpectomy procedures:
Firstly, a periapical radiograph for the involved teeth was achieved before beginning the pulpectomy procedures to confirming the diagnosis. The operative section was anesthetized with a local anesthetic drug and then isolated with rubber-dam. A previously prepared sterile kit was used during the pulpectomy procedures. Any existent caries was removed with the help of a sharp excavator or rotary handpiece with a round bur until the exposure site was identified. Deroofing of the pulp chamber and access cavity preparation was then accomplished with a fissure bur. After that, the coronal pulp tissue was eliminated with a sharp sterile excavator and then the pulp chamber was debrided with normal saline. The mechanical instrumentation of the root canals was carried out with...
rotary endodontic files until size 30, then the prepared root canals were irrigated with normal saline using an aseptic disposable 26-gauge needle and then dried with sterile paper points of convenient size [15].

**Root canals disinfected protocol:**

Following the pulpectomy procedures, the prepared root canals were disinfected carefully with the convenient disinfected protocol that was designed previously during sample grouping. In group I and group II; NaOCl and CH irritant solutions were imported carefully into the prepared root canals with 2 mm away of its working length without unwanted excessive pressure [14].

In group III; the child and operator were instructed to wear a protective eyeglass before the use of laser. Diode laser with an optical fiber of 1.5-watt power and 200μm diameter was carefully imported into the prepared root canal with a working length shorter than 1 mm from the working length of the root canal. The tip of this optic fiber was slowly moved from the apical to the coronal part of the root canal in repeated circular movements four times in raw for 15 seconds [16]. While, for group IV; the prepared root canals were irrigated with 0.2% CH solution with the same previous protocol, followed by the same protocol of laser disinfection in group III. Finally, the root canals in all groups were flushed with normal saline and dehydrated with sterile paper points of convenient size.

The pulpectomized root canals were then restored with a thick mix of zinc oxide and eugenol cement. Then the pulp chamber was filled with glass ionomer cement and finally restored with SS crown [17].

**Microbiological sampling:**

The first microbiological sample (S1) was collected immediately after access opening from each involved root canal with the help of a wet sterile paper point size 30. Each paper point was inserted into the full working length of root canals for 1 min. While the second microbiological sample (S2) was collected immediately after the use of each disinfectant protocol in such a way similar to the S1 collection [18]. Each collected paper point in S1 and S2 was immediately placed in a labeled tighten screw-capped vial containing transfer media of 2ml peptone liquid (diluent and non-selective pre-enrichment used to keep the viability of the bacteria).

The collected microbiological samples were then transported (under complete aseptic condition) to the microbiological lab as soon as possible for culture procedures on the selective Mannitol Salt Agar media. The plates with media were incubated at 37°C for 7 days, then the *S. aureus* colonies were identified microscopically, and counted, and then 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.

**IV. RESULTS:**

The results of paired t-test results revealed a significant reduction regarding *S. aureus* counting in-between the same group means after the use of each disinfectant protocol with the statistical value of p< 0.0001 (Table 1). While the statistical results of *S. aureus* baseline counting for all studied groups showed non-significant differences (p=0.917891)(Table 2). However, the results of *S. aureus* counting after the use of different disinfectant protocols showed a significant difference between groups with statistical value of (p<0.00001)(Table 2).

Among the pairwise comparison test between the groups showed a statistically significant difference in between groups (p<0.05). While, no statistically significant difference in-between (group I and III), (group II and III) with a level of significance of (p>0.05)(Table 2).

<table>
<thead>
<tr>
<th>Table 1: Comparison of <em>S. aureus</em> count (CFU/ml) before and after different disinfection protocols.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>NaOCl</td>
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Chitosan 3813.13±545.16 1701.56±237.44 < 0.0001*
Laser 3882.5±460.92 1614.44±213.89 < 0.0001*
Chitosan/laser 3926.88±559.41 1106.88±188.17 < 0.0001*

*; significant at p< 0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base-line Mean± SD</th>
<th>p-value</th>
<th>After disinfection Mean± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>3893.75±226.48</td>
<td></td>
<td>1430.63±196.08</td>
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<tr>
<td>Chitosan</td>
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Table 2: Descriptive statistics of S. aureus count (CFU/ml) along with the study.

\[ \text{NaOCl: } 3893.75±226.48, \text{ Chitosan: } 3813.13±545.16, \text{ Laser: } 3882.5±460.92, \text{ Laser/Chitosan: } 3926.88±559.41 \]

\[ \text{p-value: } < 0.0001* \]

\[ \text{Mean± SD: } 1430.63±196.08, 1701.56±237.44, 1614.44±213.89, 1106.88±188.17 \]

V. DISCUSSION:

Pulpectomy procedure is a root canal therapy that recommends as a technique for treating the necrotic or irreversibly inflamed pulp tissue of the primary teeth and is considered as a conservative treatment option instead of tooth extraction in children and young adults \cite{1,15}. During pulp therapy, the necrotic pulp tissues in the root canal were removed via mechanical instrumentation and then disinfected with irrigant solutions to eliminate the pathogenic microorganisms \cite{1,19}.

Various solutions such as NaOCl and chitosan were introduced for decreasing bacterial loading or decontaminate the infected root canals during endodontic treatment \cite{20}. In the present study, NaOCl was used with 2.5% concentration with proved antibacterial properties and less cytotoxicity \cite{21}. However, 0.2% concentration of CH was used as irrigant solution in previous studies with proved antibacterial activities \cite{22}. Also, a diode laser at a low level of power is used as a disinfectants system during the root canal treatment due to its photo-thermal ability for deep penetration inside the dential tubules \cite{18}.

In this study, S. aureus was chosen as the test microorganism because it is one of the important resistant microorganisms that is frequently isolated from primary and recurrent endodontically treated teeth \cite{7,23}. Also, the chosen culture-dependent method in this study is because the viable bacteria can easily identify on the culture medium \cite{24}.

The results of the current study showed that the baseline counting of S. aureus microorganisms of all tested groups showed no statically significant difference which indicated a standardization regarding bacterial counts in all studied groups \cite{7}. However, the finding of this study revealed a significant reduction in S. aureus colony count after using the different disinfectant protocols in all groups as indicated by paired t-test.

NaOCl irrigant solution can significantly decrease the colony count of S. aureus bacteria due to its ability to change and/or inactivate the metabolism of bacteria via the formation of chloramines \cite{25}. While CH reduces the colony count of S. aureus via its alkalinity which disturbs the cellular metabolism of the bacteria as well as its hydrophilicity which impaired the cellular permeability \cite{26,47}.

However, the mechanism of laser light to reduce the bacterial count may be due to its ability to kill this bacteriumthrough the generated heat from its intensive light \cite{18}. Moreover, the use of chitosan in combination with laser light has a synergetic effect in exiting chitosan particles and hence significantly reducing the S. aureus colony count \cite{26}.

However, the finding of this study revealed that the chitosan solution alone has lower antibacterial activity against S. aureus in comparison to other tested disinfectant protocols. This may be that S. aureus is a gram-positive microorganism \cite{26,27} that prevents the cationic groupin chitosan to bend easily with itand kill it \cite{26,28}.

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VI. CONCLUSION:

Based on the results of this study we can explore that the use of CH, and laser as disinfectant protocols alone or in combination have a significant effect in decreasing the colony count of S. aureus during pulpectomy therapy.

REFERENCE:


