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Original Research Article

Comparative evaluation of antimicrobial efficacy of QMix 2in1, Octenidine Dihydrochloride irrigants, 940nm diode laser with and without NaOCl, against mature E. faecalis biofilm: An in vitro study

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ABSTRACT

Aims: To compare the antimicrobial efficacy of NaOCl, QMix 2in1, Octenidine dihydrochloride, 940nm diode laser with and without NaOCl against matured E. faecalis biofilm.

Materials and Methods: Ninety extracted single-rooted teeth were instrumented and autoclaved. The specimens were exposed to E. faecalis contamination over a period of 21 days and subsequently divided into six distinct groups through a random assignment process. Group I-Control, Group II-NaOCl, Group III-940nm Diode laser, Group IV-940nm Diode laser + NaOCl, Group V-QMix 2in1, Group VI-Octenidine Dihydrochloride. Microbial specimens were gathered, cultured, and the quantification of colony-forming units was conducted.

Statistical analysis: ANOVA, Post hoc test, And Tuckey test were applied to find significance.

Results: 940nm Diode laser +NaOCl group showed a greater antimicrobial effect compared to other groups. No significant difference was observed between 940nm Diode laser and 940nm diode laser +NaOCl (p=.550), NaOCl and QMix 2 in 1 (p=.121), NaOCl and 940nm Diode laser groups (p =.680)

Conclusions: The concurrent use of both sodium hypochlorite and 940nm Diode laser light manifests a synergistic influence, enhancing the bactericidal efficacy. The antibacterial effect of QMix 2in1 is comparable to that of NaOCl, 940nm Diode laser, more effective than OCT and less effective than the combination of sodium hypochlorite and 940nm Diode laser.

Key Messages: A combination of 940nm laser and NaOCl is effective in reducing the bacterial count because of their capacity to penetrate effectively deep into dentinal tubules, resulting in intensified bactericidal action compared to newer irrigants used alone.

1. Introduction

The crucial goal of endodontic treatment is to eradicate bacteria and their by-products from the root canal system to achieve long-term success. E. faecalis, a predominant pathogen found in failed root canals, originates from the normal human flora and primarily exists in the form of biofilm.[1] Biofilms are 1000 times more resistant to antimicrobials, phagocytes, and antibodies when compared to isolated bacteria.[2] The primary challenge associated with traditional endodontic procedures lies in achieving adequate depth for disinfection. Microorganisms establish colonies within the dentinal tubules extending up to 1,000 μm, whereas standard rinsing solutions are limited to penetrating approximately 100 μm due to their surface tension.[3]

Utilizing diode lasers can serve as an adjunctive approach to enhancing root canal disinfection protocols.[4]

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When applied within the root canal system, diode lasers can produce diverse effects, including photothermal disinfection, stimulation of root canal irrigation solutions, and the promotion of biostimulation in the surrounding alveolar bone.[5] The sustained bactericidal effect persists due to the ability of enamel prisms and dentinal tubules to conduct light.[6]

Sodium hypochlorite (NaOCl) is commonly employed as a root canal irrigant owing to its efficient tissue-dissolving characteristics and strong antimicrobial efficacy. QMix 2in1 was shown as an effective irrigant similar to 17% ethylene diamine tetra acetic acid (EDTA) in removing the canal wall smear layer. QMix exhibited greater antibacterial efficacy against mature and nascent E. faecalis biofilms within dentin, displaying a heightened antibacterial impact specifically against E. faecalis entrenched within deep dentin layers.[1,7,8]

Octenidine dihydrochloride (OCT) (Octenisept, Schuke and Mayr gmbh, Germany) is a recently investigated solution and can be used against E. faecalis infection as an alternative irrigant. As a bispyridine derivative, it is recommended for use as an endodontic irrigant due to its reduced cytotoxicity and significant antimicrobial properties. Octenidine shows properties of high antimicrobial efficacy, positively charged chemical compounds, and a specific ability to adhere and form complexes with chemical components of cells and whole cells.[9,10]

The objective of this study was to assess the antibacterial effectiveness of a 940 nm diode laser with and without NaOCl and to compare the antibacterial efficacy of QMix 2in1 and Octenidine Dihydrochloride.

2. Materials and Methods

Ninety human single-rooted teeth displaying fully formed apices were utilized in the research investigation. Specimens were decoronated to a standard 15mm root segment length. Instrumentation of all samples was performed with Protaper universal rotary endodontic files. Irrigation was performed with a 30-gauge needle using 5ml of 5.25% sodium hypochlorite and 2ml of 17% EDTA solution. Final canal irrigation was done with 10ml of physiological saline solution. The apical opening was closed off with a light-cured restorative composite, and two coats of nail varnish were administered across the entire external surface, excluding the coronal access, to prevent external microbial contamination. Every sample was positioned in a microcentrifuge tube containing 2ml of phosphate-buffered saline (pH 7.4). Subsequently, they underwent autoclaving at 121°C and a pressure of 15 lbs for 30 minutes.

A culture containing solely Enterococcus faecalis (ATCC 29212) obtained from Microbiologics in India was utilized to contaminate the root canals. The suspension was created by combining 1 ml of culture in brain heart infusion (BHI) broth (obtained from Titan Biotech in India) and allowing it to incubate for 24 hours. The McFarland standard number 0.5 was employed to assess the broth, ensuring a bacterial count close to 1.5 x 10^8 colony-forming units (CFU) per ml [Figure 1c].

Each of the specimens was relocated into individual sterile microcentrifuge tubes. A micropipette calibrated to 10 microlitres was used to carry BHI broth into prepared root canals. After inoculation, the tubes were incubated at 37^oC for 21 days [Figure 1a]. At intervals of three days, a 0.1ml sample was extracted, followed by the addition of 0.5ml of fresh BHI broth to maintain bacterial viability. Subsequently, the canals were dried using sterile paper points after the incubation period.

The samples were randomly assorted, leading to the establishment of a single control group alongside five experimental groups (n=15) based on the irrigant used. This entire procedure was done under aseptic conditions in a laminar airflow chamber.

Group A: In the control group, after drying the canals with paper points, the root canals were irrigated with saline.

Group B: NaOCl, The specimens underwent irrigation using a 5% NaOCl solution totaling 3 ml, administered over a period of 1 minute.

Group C: 940nm diode laser, Epic X from Biolase in San Clemente, California, USA, was utilized for laser treatment. The endodontic tip employed was the ezTip Endo, measuring 14 mm in length and 200 μm in diameter, operating at a wavelength of 940 nm. The treatment was carried out following the manufacturer's instructions, utilizing a pulse interval of 0.2 ms in a repeated pulse mode with a pulse duration of 0.05 ms and an output power of 3.5 W. The laser irradiation was directed into the canal, ceasing 1 mm before reaching the working length, and continued for 1 minute. It involved circular movements starting from the apical part towards the coronal part, following the step-back technique, which entailed four exposures of 15 seconds each, with a 20-second dwell time between exposures. The laser probe was directly inserted into the root canal during the procedure.

Group D: 940nm diode laser with NaOCl, the irrigation procedure was performed similarly to group B. After irrigation was done with 5% NaOCl, laser irradiation was performed directly in the remaining NaOCl solution, as in group C.

Group E: QMix 2in1, specimens were irrigated with 3 ml of QMix 2in1 for 1 minute.

Group F: Octenidine Dihydrochloride, for preparation of octenidine dihydrochloride irrigant, 100 mg of powder is mixed in 100ml of distilled water under aseptic conditions to prepare 0.1 % solution. Specimens were irrigated with 3ml for 1 minute.

In all groups, irrigation was carried out using a 30-gauge side-vented needle, passively inserted up to 2 mm

from the working length. Subsequently, a paper point was introduced into the specimens to desiccate the root canals for 30 seconds, followed by the introduction of a sterile saline solution into the specimens.

The sampling procedure within the canals involved employing a sterile #25 H-File with circumferential filing for 20 seconds to disturb the biofilm and gather dentin chips. An inoculation loop of 2mm diameter was used to collect BHI broth containing microbial suspension [Figure 1b], inoculated on blood agar plates and incubated for 24 hours at 37^0C. The quantification of bacterial colonies was conducted, and the findings were reported in CFU/ml (colony-forming units per milli liter). Descriptive statistics were presented using the mean accompanied by the standard deviation (SD). To determine significance, ANOVA and subsequent post hoc analysis through the Tukey test were employed. A statistical significance was established at the level of $p<0.05$.

3. Results

Under the experimental conditions of the current in vitro study, the results showed that none of the irrigants or laser irradiation had eliminated colony counts in the root canal. However, the highest bacterial count was seen in Group A, followed by Group F and the lowest count was observed in Group D [Figure 2]. An intergroup comparison for experimental groups utilizing the post hoc Tukey test revealed a statistically significant difference in colony-forming units between all groups except Group C and Group D, Group B and Group E, and Group C and Group B [Table 1].

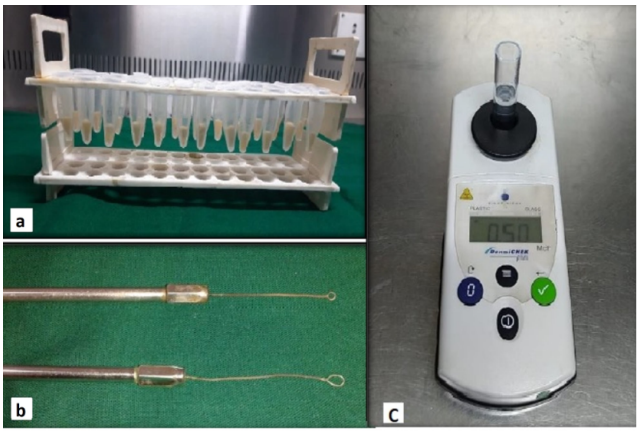

Figure 1: a: Samples inoculated with Enterococcus faecalis and incubated; **b**: Inoculation loop used to collect BHI broth containing microbial suspension and transfer to culture plates; **c**: Bacterial suspension adjusted to 0.5 Mc Farland units.

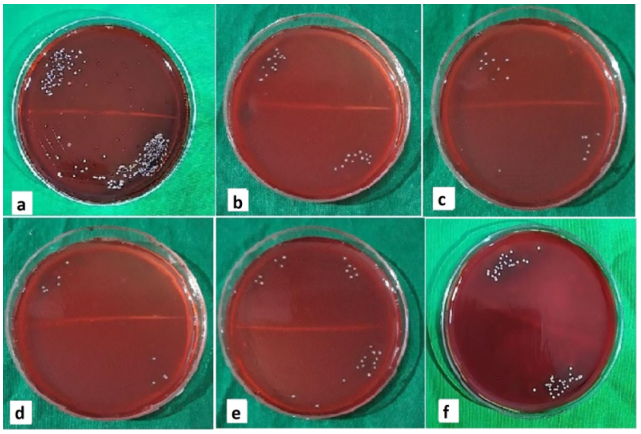

Figure 2: Culture plates showing bacterial growth of (**a**) Group A: Control, (**b**) Group B: NaOCl (**c**) Group C: 940nm diode laser (**d**) Group D: 940nm diode laser with NaOCl (**e**) Group E: QMix 2in1 (**f**) Group F: Octenidine Dihydrochloride.

Table 1: Intergroup comparison of experimental groups

Intergroup Comparison		p-value
NaOCl	940nm DIODE LASER	.680
NaOCl	940nm DIODE LASER +NaOCl	.026
NaOCl	QMix 2 in 1	.121
NaOCl	OCT	<0.001
940nm DIODE LASER	940nm DIODE LASER + NaOCl	.550
940nm DIODE LASER	QMix 2 in 1	.002
940nm DIODE LASER	OCT	<0.001
940nm DIODE LASER + NaOCl	QMix 2 in 1	<0.001
940nm DIODE LASER + NaOCl	OCT	<0.001
QMix 2 in 1	OCT	<0.001

4. Discussion

Various bacteriological investigations indicate that E. Faecalis is detected in 30% to 48% of teeth experiencing post-treatment infections.[4] It has been reported that high alkaline tolerance, genetic polymorphism, long-term starvation, and the presence of protein binding properties help in adhesion to dentin and its retention in the dentinal tubules.

Microorganisms can survive under extreme environmental conditions because of the biofilm.[11] Biofilm gives them higher resistance to antibacterial agents. Therefore, as closely as possible to mimic in vivo conditions, the biofilm model was used in the present study.[12] In our experiment, the contact duration between the biofilm and irrigant was usually set at 1 minute, allowing for comparison under in vivo conditions, as opposed to

longer durations of 15 or 60 minutes.[2]

NaOCl was used as a test irrigant because of its antibacterial activity and effective tissue dissolution properties.[13] The results showed that the mean colony was $2.28x10^3$. The results were in accordance with Fidalgo et al. (2010),[14] who concluded that 5.25% NaOCl is a microbiocide, whereas 0.5% and 1% NaOCl are only microbiostatic against the tested E. faecalis. However, it showed less antibacterial efficacy when compared to laser groups in the present study.

Group C showed a mean colony forming units 1.69×10^3. It showed superior results when compared to other groups except for Group D. The reason may be that laser light may not uniformly access all regions as effectively as sodium hypochlorite rinsing does. One limitation associated with laser fibres is the requirement for a helical movement within the root canal to expose the surface to the laser light in areas that might not be exposed to the laser beam or not have been exposed sufficiently.[4]

Group D showed mean colony-forming units of $1.0^3 \times 10^3$. This result could arise from the combined effect of both the laser and sodium hypochlorite in addition to the laser's capability to increase the temperature of the hypochlorite solution.[4] Benedicenti et al. (2008)[15] showcased the combined synergistic impact of employing citric acid, diode laser energy, and NaOCl, resulting in notably enhanced root canal decontamination and heightened bactericidal efficacy. Preethee et al. (2012)[16] Confirmed an enhanced bactericidal efficacy when using a combination of diode laser and irrigants compared to canals disinfected solely with the diode laser.

Mehrvarzfar et al. (2011)[17] proposed a combination approach involving chemical irrigation and laser treatment as an efficacious option for eradicating E. faecalis from the root canal system. Rahimi et al. (2012)[18] stated that the efficacy of the laser alone in root canal disinfection is inferior to its combined usage with NaOCl; therefore, employing the laser alongside root canal irrigants was advised.

Diode lasers demonstrate significant water penetration capability, enabling interaction with microorganisms situated within the deeper layers of dentinal tubules. This feature results in a remarkably potent bactericidal effect, particularly effective against highly resistant endodontic pathogens, even at depths reaching 1000 μm within the dentine.[19] The use of diode laser irradiation leads to the elimination of the smear layer, disinfection, as well as the fusion and closure of dentin tubules. This process enhances the sealing capability of the root canal, especially in the apical third region, thereby reducing the likelihood of reinfection.[20]

In this study, the Group E results showed that the mean CFU of QMix 2 in 1 was 3.29×10^3. This is in accordance with the study done by Wang et al., who determined that QMix and 6% NaOCl exhibited more potent antibacterial effects against both young and old E. faecalis biofilm in dentin compared to 2% NaOCl and 2% CHX.[21] Chandrasekhar V. et al. (2013)[22] stated that QMix demonstrates comparable effectiveness to EDTA in eliminating the smear layer and exhibits greater biocompatibility in comparison to numerous other irrigants.

In this study, NaOCl and QMix 2 in 1 showed superior results when compared to OCT; this may be due to less contact time with the root canal walls. This is supported by the study done by Tandjung et al. (2007),[23] who concluded that OCT demonstrated effectiveness after a 10-minute incubation, displaying a notable reduction. Their findings suggest that the utilization of OCT as a root canal medicament for a prolonged duration proved to be effective.

Further research on OCT, including its interactions with other irrigants, dose-effectiveness, optimal regimens, organoleptic properties, and modes of action, such as the antibiofilm effect and antimicrobial efficacy against other singles or multispecies biofilms, are required. Subsequent studies employing multispecies biofilm within intricate root canal systems are necessary to substantiate and reinforce these conclusions.

Under the limitation of the present in vitro study, it was found that none of the irrigants or laser groups used had eliminated the bacteria within the root canal system. Nonetheless, there was a noteworthy decrease in the bacterial count.

The order of bacterial count was as follows: Group A (Control) > Group F (OCT) > Group E (QMix 2 in 1) > Group B (NaOCl) > Group C (940nm Diode laser) > Group D (940nm Diode laser + NaOCl). Hence, it can be concluded that the combination of sodium hypochlorite and 940nm Diode laser light can intensify the bactericidal action by having a synergistic effect.

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None.

6. Conflict of Interest

None.

7. Acknowledgement

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