ORIGINAL RESEARCH

Comparison of Antimicrobial Efficacy of Diode Laser, Ultrasonic Activated and Conventional Irrigation with 2.5% NaOCl during RCT: An Interventional Study

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Abstract

Aim and objective: The aim and objective of the study was to evaluate and compare the antimicrobial efficacy of a diode laser irradiation, ultrasonic activated and conventional irrigation with 2.5% NaOCl on obligatory and facultative anaerobic bacteria in single-rooted canals.

Materials and methods: Total of 60 permanent maxillary and mandibular single-rooted (single canal) anterior teeth were selected. First microbial sample (S1) was collected after access opening and working length determination, using a sterile paper point. Cleaning and shaping were performed, with each instrument change accompanied by irrigation using 2 mL 2.5% NaOCl. After cleaning and shaping, disinfection protocol using diode laser (group1), ultrasonic activated irrigation with 2.5% NaOCl (group 2) and conventional irrigation with 2.5% NaOCl (group 3) was performed and second microbial sample (S2) was obtained. The colony characters of each type of growth on each media were noted and the organisms were identified using standard biochemical reactions.

Result: Gram-positive and gram-negative facultative anaerobe were predominantly isolated from the culture, and the highest reduction of the microbial count was seen in diode laser group with 60.92% followed by the ultrasonic group with 47.22% reduction and least reduction was observed in conventional irrigation with the ultrasonic group with 37.97%. The results were statistically significant with *p*-value <0.05.

Conclusion: Diode laser disinfection showed the highest reduction of microbial count compared to ultrasonic activated and conventional needle irrigation with 2.5% NaOCl group.

Clinical significance: This study will help us to choose wisely between various irrigating methods and protocols. Diode laser in our study has shown superior disinfection of the root canals compared to others.

Keywords: 2.5% NaOCl, Conventional needle irrigation, Diode laser intracanal disinfection, Ultrasonic irrigation.

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Introduction

Endodontic infections are known to be polymicrobial in nature, with complex bacterial interactions and a predominance toward anaerobic species.[1] A major goal of endodontic treatment is to achieve a bacteria-free environment in the root canal system to prevent any menace for successful treatment.[2]

Elimination of endodontic infection is quite different from most of the other sites in the human body. Host measures that are sufficient to eliminate the infectious organism from other sites do not suffice for the complete elimination of endodontic infection, mainly because of special anatomy and physiology of root canal.[3] Large areas of the root canal such as fins, isthmus, cul de sac remain untouched even after mechanical instrumentation, regardless of the use of rotary or hand instrumentation. For this reason, various combinations of disinfecting solutions and various irrigation devices are used.[4]

Sodium hypochlorite (NaOCl) is one of the most widely used irrigating solution, hypochlorous acid in NaOCl is considered responsible for bacterial inactivation, when used in concentration varying from 0.5 to 6% effectively dissolves pulpal remnants and organic components of dentin.[5]

Irrigants have been traditionally delivered using a syringe and needle. The problem with this method of irrigation technique is an inadequate replacement of irrigant through the root canal system because the highest streaming velocity is present only in the lumen of the needle and high surface tension of NaOCl prevents direct contact with dentinal walls of anatomic complexities.[6]

Over the last few decades, many mechanical devices have been developed to improve the penetration and effectiveness of irrigating solutions in the apical areas of root canal space. Passive ultrasonic irrigation (PUI) was first described by Weller et al. in 1980. It is called passive because it is related to the noncutting action of ultrasonic files. PUI relies on the transmission of acoustic energy from an oscillating file to an irrigant in the root canal. The energy is transmitted by means of ultrasonic waves.[4]

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Relatively new approaches to root canal disinfection include high power diode laser which made their debut in mid 1900s in the field of dentistry which has been predominantly used for soft tissue surgery, root canal disinfection, and laser-assisted bleaching.[7] The available wavelength ranges from 800 to 1064 nm, which emit continuous wave or gated pulsed mode using an optical fiber as a delivery system. A combination of smear layer removal, bacterial reduction, reduced apical leakage are advantages of the laser and make it viable for endodontic treatment.[4] The laser light is thought to be able to reach areas in the root canal anatomy, which is impossible with traditional techniques.[3]

When the literature search was carried out, there were no studies carried out in Indian scenario or worldwide to evaluate the antimicrobial efficacy of a diode laser irradiation, ultrasonic activated and conventional irrigation with 2.5% NaOCl on obligatory and facultative anaerobic bacteria in single-rooted canals. So, an attempt was made to carry out this research to evaluate the antimicrobial efficacy of a diode laser irradiation, ultrasonic activated and conventional irrigation with 2.5% NaOCl on obligatory and facultative anaerobic bacteria in single-rooted canals.

Materials and Methods

The study was conducted in the KLE's VK Institute of Dental Sciences, Belagavi, Karnataka. Ethical clearance was obtained from the Research and Ethical Committee of KLE Academy of Higher Education and Research (KAHER), Belagavi.

Sixty maxillary and mandibular single-rooted canals (permanent incisors, laterals, canines, premolars), of both male and female patients of the age range of 15–50 years, with written informed consent were selected for the study. Patients were recruited from the out-patient department of conservative dentistry and endodontics in accordance with specific inclusion and exclusion criteria and sequential allocation of subjects was done into one of the three groups. A written informed consent was obtained from all participants before the allocation.

The inclusion criteria used in our study are patients with permanent teeth diagnosed as nonvital clinically using pulp vitality tests and radiographs, participants with noncontributory medical history, teeth with an adequate coronal structure to ascertain proper isolation, sterilization, temporization, and restoration. Exclusion criteria used in the study are patients with any systemic diseases, immune-compromised conditions and pregnancy, participants on any antibiotic therapy within 3 months from the beginning of the study, patients with acute periapical abscess, retreatment cases, teeth with calcified canals, immature apex and internal and external resorption were excluded from the study.[8]

Each tooth to be treated was anesthetized using local anesthesia (Xicaine, ICPA Health Product Ltd, Mumbai, India), isolated with a rubber dam (Hygenic, Coltene, Altstatten, Switzerland), and tooth crown cleaned with pumice and rinsed with sterile saline (Baxter India Limited, Bengaluru, India). The operative field was then disinfected following Mollers methodology of disinfection.[9]

Pulp chamber was opened under aseptic conditions with sterile water-cooled high-speed diamond points (Mani Dia-burs, Tochigi, Japan). Working length was determined by the apex locator (Dentaport ZX, J Morita, Tokyo, Japan) and confirmed on the radiographs. The first root canal sample S1 was obtained as follows.

Reduced transport fluid (transport media) was injected into the root canal and a size 10 or 15 K-file (MANI; Japan) was pumped circumferentially to 1 mm short of the estimated working length. A sterile paper point (Protaper Universal, Dentsply, Switzerland) was then inserted into the canal 1 mm short of the estimated working length and kept in place for 60 seconds. Three paper points were taken for each sample following the same procedure as described above and each of the paper points was immediately transferred to a reduced transport fluid (transport media).[10] The sample S1 was collected and transferred to the laboratory immediately.

Thereafter, in the next appointment, instrumentation was performed with ProTaper Universal rotary system (Dentsply, Switzerland) using the crown down technique. A 2.5% sodium hypochlorite was used for irrigation in all three groups which were prepared from 3% NaOCl (Vishal Dentocare, Gujrat, India) by the Alligation method.[11]

After cleaning and shaping, root canals were divided into two experimental and one control group by sequential allocation of subjects into one of the three groups.

- **Group I:** The root canal was disinfected with high power diode laser 940 nm (Epic Biolase, California, USA) set at power 1 watts, using an oscillatory technique, the diode fiber with 200 µ fiber-optic tip was introduced 1 mm short of apex, for 5 seconds, and repeated four times at an interval of 5 seconds between each one.
- **Group II:** The root canal was ultrasonically irrigated (P5 Booster, Suprasson, SATELEC, France) for 3 minutes with a continuous flow of 50 mL with 2.5% sodium hypochlorite.
- **Group III:** The root canal was irrigated with 2 mL of 2.5% sodium hypochlorite, after each instrumentation with a disposable syringe of 26 gauge needle (Unolok, Hindustan Syringes and Medical Devices Pvt. Ltd, Faridabad, India) and the second sampling (S2) were carried out above all groups.

After the collection of sample S2, the final flush of irrigation was done with 2 mL of 17% EDTA (Prime Dental Product, India). Finally, the canals were rinsed with sterile saline followed by obturation.

For the microbiological procedures of sample S2, the clinical samples were collected in reduced transport fluid (transport media) and transferred to the laboratory immediately. The microbial analysis was done by counting the colony forming units (CFUs).

Microbiological Procedure

For microbiological procedures, the clinical samples were collected in reduced transport fluid and transferred to the laboratory immediately. Test tubes containing samples were incubated for 30 minutes at 37°C and shaken vigorously in vortex mixture for 60 seconds. Before inoculation samples were subjected to serial tenfold dilution in brain heart infusion broth. From the serial dilution, 0.1 mL was transferred to the culture media with 5.0 µg/mL hemin and 0.5 µg/mL menadione.

For Obligate Anaerobes

The inoculum was mixed thoroughly and inoculated on supplemented Blood agar, Kanamycin Blood agar, and Brewer Anaerobic agar. This was incubated in a modified gas pack anaerobic jar for 5 days. Once the jar was opened the colony characters were studied and each colony type was immediately inoculated in thioglycollate broth. The final identification of the organism was carried out using a standard biochemical reaction. Separate selective media Actinomyces agar was prepared for *Actinomyces* species and incubated anaerobically as described previously.

For Facultative Anaerobes

Ten microliters of the sample was transferred to each of Chocolate agar, MacConkey's agar, and Mitis Salivarious agar (MSA). The first two media were incubated aerobically at 37°C for 24 to 48 hours. The MSA was incubated in carbon dioxide jar (5% Carbon dioxide) for 48 hours. For *Candida* species, Sabourauds Dextrose agar was used. The colony characters of each type of growth on each media were noted and the organisms were identified using standard biochemical reactions.

Results

Individual root canals yielded few anaerobic bacterial species, gram-positive and gram-negative facultative anaerobe were predominantly isolated from the culture. The species identified along with the percentages have been recorded and tabulated in Table 1.

For the purpose of ease of statistical analysis, the CFUs counts were converted into logarithmic transformation (log CFU).

In laser group, it was found that the log CFU value at the baseline level (S1) was 4.25 ± 0.08 which reduced to 1.66 ± 0.35 postoperatively (S2). The difference was statistically significant with the *p*-value of 0.00001* and the percentage of change seen from baseline to postoperatively was 60.92% (Table 2, Fig. 1).

Table 3 and Figure 2 shows comparison made between S1 and S2 value in ultrasonic group, the baseline level (S1) found was 4.25 ± 0.65 which reduced to 2.45 ± 0.40 postoperatively (S2). The difference was statistically significant with the *p*-value of 0.0002* and the percentage of change seen from baseline to postoperatively was 42.22%.

Table 1: Species identified from the root canal along with their percentages

Sl. No.	*Microrganism*	*Type*	*Percentage*
1	*Streptococci*	Gram-positive facultative anerobe	49.14
2	*Escherichia coli*	Gram-negative facultative anaerobe	1.04
3	*Fusobacterium nucleatum*	Gram-negative obligate anaerobe	0.63
4	*Porphyromonas gingivalis*	Gram-negative obligate anaerobe	0.80
5	*Proteus* species	Gram-negative facultative anaerobe	9.04
6	*Klebsiella*	Gram-negative facultative anaerobe	35.88
7	*Micrococci*	Gram-positive facultative anaerobe	0.17
8	*Pseudomonas* sp.	Gram-negative facultative anaerobe	0.62
9	*Diptheria*	Gram-positive facultative anaerobe	0.44
10	*Citrobacter*	Gram-negative facultative anaerobe	1.69
11	*Provetella intermedia*	Gram-negative obligate anaerobe	0.23
12	*Shigella cocci*	Gram-negative facultative anaerobe	0.26

Table 2: Table showing comparison of S1 and S2 with respect to log CFU counts in group I (diode laser) by paired "*t*" test

Time-points	*Mean*	*Std. Dv.*	*Mean Diff.*	*SD Diff.*	*% of change*	*Paired t*	*p-value*
S1	4.25	0.34					
S2	1.66	1.58	2.59	1.54	60.92	7.5307	0.00001*

Table 3: Table showing comparison of S1 and S2 with respect to log CFU counts in group II (ultrasonic group) by paired "*t*" test

Tim-points	*Mean*	*Std. Dv.*	*Mean Diff.*	*SD Diff.*	*% of change*	*Paired t*	*p-value*
S1	4.25	0.65					
S2	2.45	1.78	1.79	1.75	42.22	4.5912	0.0002*

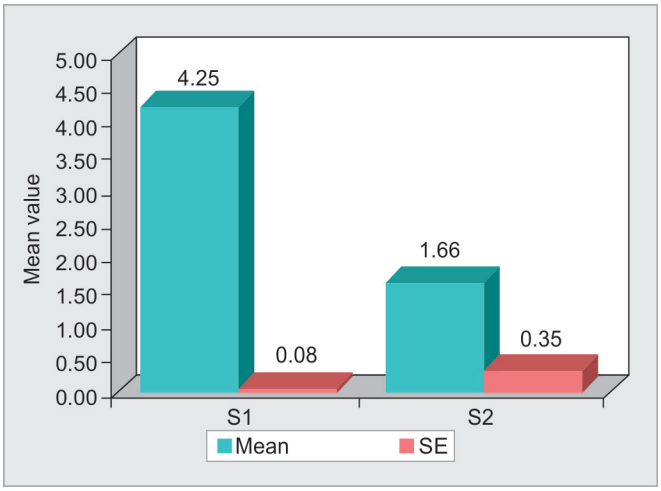

Fig. 1: Graphical representation showing comparison of S1 and S2 with respect to log CFU counts in group I (diode laser) by paired "*t*" test

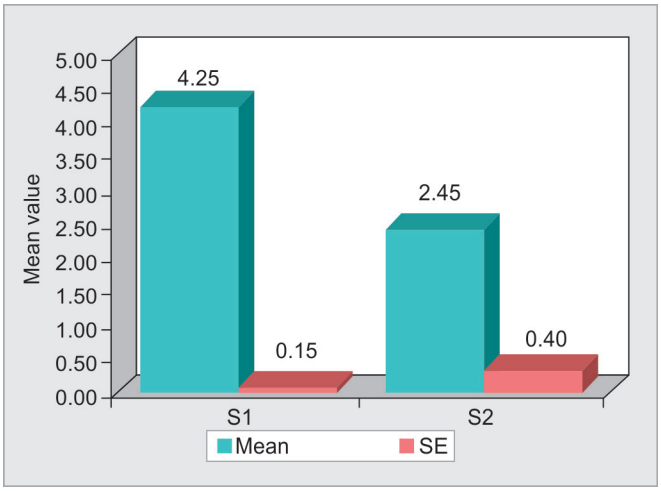

Fig. 2: Graphical representation showing comparison of S1 and S2 with respect to log CFU counts in group II (ultrasonic group) by paired "*t*" test

Table 4: Comparison of S1 and S2 with respect to log CFU counts in group III (conventional needle irrigation group) by paired "*t*" test

Tim-points	*Mean*	*Std. Dv.*	*Mean Diff.*	*SD Diff.*	*% of change*	*Paired t*	*p-value*
S1	4.00	0.90					
S2	2.48	1.89	1.52	1.84	37.97	3.6963	0.0002*

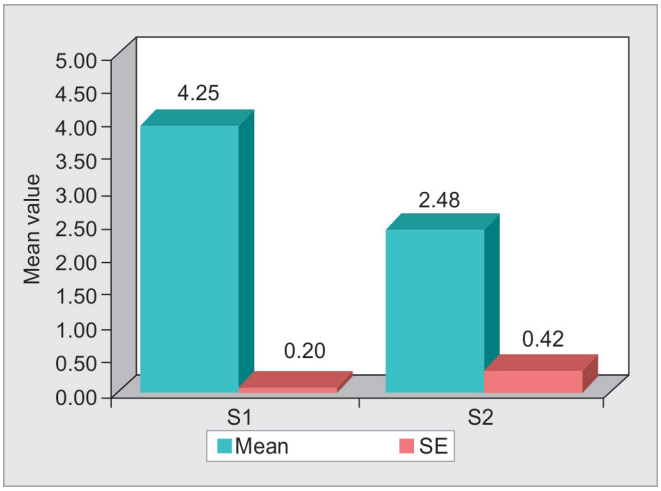

Fig. 3: Graphical representation showing comparison of S1 and S2 with respect to log CFU counts in group III (conventional needle irrigation group) by paired "*t*" test

In conventional needle irrigation group, it was found that the log CFU value at the baseline level (S1) was 4.00 ± 0.20 which reduced to 2.48 ± 0.42 postoperatively (S2). The difference was statistically significant with the *p*-value of 0.0002* and the percentage of change seen from baseline to postoperatively was 37.97% (Table 4, Fig. 3).

Inference

Highest reduction of the microbial count was seen in diode laser group with 60.92% followed by ultrasonic group with 47.22% reduction and least reduction was observed in conventional irrigation with ultrasonic group with 37.97%.

Discussion

Microorganisms and their end products are considered as the main cause of pulpal and periapical pathosis. The prime objective of endodontic therapy is the complete elimination of microorganisms from the root canal system and to create an environment favorable for healing. Bacterial elimination from the root canal is usually achieved by means of mechanical action of the instruments along with flushing and antibacterial activity of the irrigants.

Over the years, disinfection protocols have evolved from conventional needle irrigation, sonic and ultrasonic agitated irrigation, ozone disinfection, photoactivated disinfection (PAD) to the more recently introduced laser-activated disinfection. Sodium hypochlorite is the most widely used root canal irrigant, which disrupts several vital functions of microbial cells, causing cell death.[12]

Although studies conducted by Vianna et al. and Gomes et al. showed that a high concentration of 5.25% NaOCl was more effective and required less time to eradicate microorganisms. However such high concentration of NaOCl is associated with hemolysis and necrosis of tissues when inadvertently extruded beyond the root apex.[13] Bystrom and Sandquist observed no significant difference in concentration of 1, 2.5, and 5% NaOCl in its antibacterial effectiveness[14] which goes with the results of Zehnder, that the effectiveness of 5% NaOCl was not any greater than the lower concentration in reducing the intracanal microbiota; therefore, a lower concentration of 2.5% NaOCl was used.[15]

In the present study, it was found that the conventional needle irrigation group was inferior to the ultrasonic irrigation and diode laser group and the results were statistically significant. The bacterial reduction of 37.97% was achieved in the conventional needle irrigation group. This inferior performance of conventional needle irrigation was similar to observations made by Bago et al., which showed that conventional needle irrigation was not successful in eliminating microorganisms from the root canal when compared to sonic activated irrigation and PAD.[4]

Inadequate placement of irrigant in the root canal system can be one of the reasons as the highest streaming velocity is only present in the lumen and around the tip of the needle making NaOCl difficult to reach inaccessible areas. The high surface tension of NaOCl also prevents the irrigant from coming in direct contact with dentinal walls of anatomical complexities.[3] Studies also showed that the irrigant has only a limited effect beyond the tip of the needle because of the dead-water zone or sometimes air bubbles in the apical root canal, which prevent apical penetration of the solution.[16]

PUI as described by Ahmad et al. enhances the flushing action for removal of organic and inorganic debris from the root canal walls. The hydrodynamic phenomenon caused by vibration of the file in the canal filled with irrigant causes acoustic microstreaming, which is responsible for the removal of debris and bacteria from the dentinal tubules.[17]

In the present study, it was found that the ultrasonic irrigation group was superior to conventional needle irrigation and this difference was statistically significant. A bacterial reduction of 42.22% was achieved in ultrasonic irrigation group. The result of our study was similar that to the study conducted by Brito et al., where a significant reduction in the CFU count was observed after 1 minute of ultrasonic agitation. The higher performance of ultrasonic agitation of irrigant over sonically activated irrigation could be due to higher frequency of cavitation resulting in higher acoustic streaming of the former.[14]

Kim reported similar results in which PUI was better than conventional needle irrigation in the removal of pulp remnants and dentin debris. This could be due to the flow of the irrigant at a high velocity which was achieved due to passive ultrasonic activation thus facilitating the removal of debris from the root canal irregularities and oval-shaped canals.[14] The reduction in the number of microorganisms could also be due to deagglomeration of the bacterial biofilms within the root canal which may render the resultant planktonic bacteria more susceptible to the antibacterial activity of NaOCl.[4]

Ultrasonically activated irrigation has shown low efficacy as compared to laser and the factors governing these results can be the unknown variables involved such as placement of the suction, the width of irrigation jet, and location and dimension of root canal orifice, amount of irrigant flowing through the canal.[15]

In a review by Sluis et al., the protocol for using ultrasonic irrigation was influenced by the taper of the file and diameter of the root canals. Greater the taper of the file more dentin debris could

be flushed out of the root canal. Other factors influencing debris removal were the time of irrigation and volume of irrigant.[16] Neto et al. suggested that 5 minutes of ultrasonic irrigation removed more dentin debris than 1 minute of the continuous flow of NaOCl when volumes were similar in both the groups.[17]

In the present study, it was found that the diode laser group was superior to conventional needle irrigation and this difference was statistically significant. The bacterial reduction of 60.92% was achieved in the diode laser group. The diode laser group was more efficient followed by the ultrasonically activated group and least microbial reduction was observed in conventional needle irrigation with 2.5% NaOCl. The results obtained in the present study were in accordance with observations made by Mathew et al. where disinfection with 940 nm diode laser was far superior in reduction of the microbial count when compared to sonic irrigation and conventional needle irrigation groups.[18]

Since the size of the dentinal tubules progressively decreases apically the penetration of the irrigant becomes restricted.[19] Laser with its inherent property of light scattering, enhanced local intensity and attenuation allows light penetration deeper in dentinal tubules, contributing to its superior antimicrobial efficacy. Diode laser causes a thermal photo disruptive action in the untouched part of dentin, resulting in an enhanced bactericidal effect in the root canal dentin.[9] Fine diameter of 200 µm fiber-optic tip was used in our study which facilitated the effective delivery of laser light to the root canal walls and effective reduction in the bacterial count. Small fiber-optic increased the power density of the tip and also gained easy access to apical third thereby extending its use to effectively debride canals with curvature.

The present study could not 100% eliminate the microbes. The primary reason could be due to the resistance of *Enterococcus faecalis* to heat produced by laser attributed to its complex cell wall structure.[2] Failure could also be due to the emission of laser energy from the tip of the optical-fiber which was not lateral onto the root canal walls making it impossible to obtain uniform coverage of the canal surface using laser.[8]

In this study, the high-power diode laser and ultrasonic irrigation were superior to conventional needle irrigation with 2.5% NaOCl in eliminating intracanal microorganisms. However, diode laser disinfection and ultrasonic irrigation cannot replace shaping, cleaning, smear layer, and biofilm management but could go hand in hand with a perfect routine endodontic treatment in order to improve the outcome. Mandatory to a benefit of adjunctive diode laser irradiation treatment and ultrasonically activated irrigation, all steps of the routine endodontic treatment should be followed carefully.

Conclusion

All the groups showed a significant reduction in the microorganisms suggesting these disinfection methods can successfully be used *in vivo*. However, diode laser disinfection showed the highest reduction of microbial counts compared to ultrasonic activated and conventional needle irrigation with 2.5% NaOCl group. Diode laser disinfection and ultrasonically activated irrigant can only be used as an adjunct to conventional needle irrigation with NaOCl.

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