DOI: 10.7860/JCDR/2014/8412.5215

Original Article

Dentistry Section

Bacterial Efficacy of $Ca(oH)_2$ Against *E.faecalis* Compared with three Dental Lasers on Root Canal Dentin- An Invitro Study

NARASIMHA REDDY KANUMURU[1], RAMA SUBBAIAH[2]

ABSTRACT

Aim: The aim of this study was to evaluate bactericidal effect of $Ca(OH)_2$ compared with 810 nm diode, 980 nm diode, and Nd:YAG lasers on root canal dentin against *E.faecalis*.

Materials and Methods: Sixty five freshly extracted human mandibular single rooted teeth were selected for the study. The apical third of these roots was gradually enlarged until reaching the ISO 40- K file. The samples were divided into 4 groups, each containing 15 teeth and 5 teeth for control group. Group-1: 810 nm Diode; Group-2: 980 nm Diode; Group-3: Nd:YAG; Group-4: $Ca(OH)_2$. 50µL of the *E.faecalis* ATCC 29212 strand was incubated in 1 mL of Brain Heart Infusion Broth (BHI) culture medium in37°C incubator for 4h. The concentration of the inoculation was then adjusted for a degree of turbidity which was adjusted to 0.5 McFarland scale. Later from the incubated broth, 10µL of *E.faecalis* culture was inoculated into the main canal and were sealed.

Results: The incubated plates were checked for growth and the colony was counted using colony counter and the results are interpreted. There was statistically significant difference ($p<0.05$) amongst the $Ca(OH)_2$ group regarding the laser groups.

Conclusion: The teeth irradiated with the Nd:YAG laser had significantly higher bacterial reduction than all the other groups and the respective control groups.

Keywords: Diode laser, $Ca(OH)_2$, *E.Faecalis,* Nd:YAG laser

INTRODUCTION

Enterococcus faecalis, a facultative anaerobe, has been commonly isolated from persistent root canal infections and post-treatment infection. One of the virulence factors associated with this persistent presence of *E.faecalis* in root canals is its ability to invade dentinal tubules, adhere to collagen in the dentine. An in-vitro study has shown that albeit with the presence of intracanal medications, the *E.faecalis* was able to form biofilm in the root canals. Biofilm is a highly organized structure with clumps of bacteria bound together by a carbohydrate matrix. In addition, the *E. faecalis* has the ability to survive without dividing in the biofilm and induced the apatite reprecipitation, especially in a mature biofilm [1,2].

Hence, these features explain the low susceptibility of *E. faecalis* biofilm towards several antimicrobial agents when compared to its other morphotypes such as the planktonic and pellet forms. The endodontic microflora is polymicrobial and the established lesion has a preponderance of gram negative anaerobes. These bacteria will be very resistant to chemo mechanical debridement and oxygenating agents like hydrogen peroxide and sodium hypochlorite. Failure to effectively eliminate the bacteria and their by-products might result in persistent irritation and impaired healing. It has been widely reported that viable bacteria can remain within the canal system even after chemo mechanical preparation[1,2].

Several studies have shown that the *E. faecalis* is highly resistant to the antibacterial effect of calcium hydroxide. reported that *E. faecalis* can survive in an alkaline environment as high as pH 11.1 and could only be killed when pH reached higher than 11.5. However, the pH of the $Ca(OH)_2$ in the canal could only reach up to 10.3 due to the buffering effect of the radicular dentin Evans et al., [3,4].

Normal irrigants were used for disinfection have many disadvantages due to smear layer present on root canal dentin. The introduction of lasers in root canal therapy has improved to eliminate bacteria from deep dentinal tubules [3,4].

AIM AND OBJECTIVES

The aim of this study was to evaluate bactericidal effect of $Ca(OH)_2$ compared with 810 nm diode, 980 nm diode, and Nd:YAG lasers on root canal dentin against *E.faecalis*.

MATERIALS AND METHODS

Sixty five freshly extracted human lower single rooted teeth were selected for the study (3 months). The samples were collected from the Department of Oral surgery, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India. They were stored in saline until use. First the crowns were decoronated at the cement enamel junction, using diamond disc at medium speed. The coronal third of the canals were enlarged using Gates Glidden (GG) burs (no.3). The root canals were instrumented to the maximum apical file size of #40 by step back technique. The root canals were thoroughly rinsed with saline solution. After instrumentation the canals were irrigated with EDTA followed by 2.5% NaOCl solution for 10 min. Then the apical third of roots were resected so that each specimen measured to be 6 mm. The samples were then sterilized in an autoclave for 15 min at 121°c in 15 lbs. The samples were then divided into 4 groups, each containing 15 teeth and the remaining 5 teeth were allocated for control group.

Group-1: $Ca(OH)_2$

Group-2: low power diode laser (810nm)

Group-3: high power diode laser (980nm)

Group-4: Nd:YAG laser

Selection and Preparation of Bacteria

The smear layer of the root canal was removed by treating the tooth sections in an ultrasonic bath of 17% EDTA. A pure bacterial culture of the Gram-positive cocci *Enterococcus faecalis* (ATCC 29212) was obtained. The grown bacterial colonies were then harvested and placed in Brain Heart Infusion Broth and incubated for 24 h at 37°C under aerobic conditions. The turbidity of BHI containing *E. faecalis* was adjusted to McFarland 0.5 standard which corresponds to 1.5×10^8 colonies. Then the 20 µL of the bacterial culture was transferred into the canal lumen of the mechanically enlarged root canals using a sterile micropipette and stored at 48 h at 37°C.

L**aser Irradiation:** An Nd: YAG laser system operating at a wavelength of 1.064 nm with a pulse duration of 100 µsec in single pulse mode was used. A Diode laser device emitting radiation at a

Groups	Before Treatment CFU/ML	Before Treatment SD	After Disinfection CFU/ML	After Disinfection SD
GROUP-1 $Ca(OH)_2$	1.12×10^8	35.0	0.5×10^4	8.31
GROUP-2 810nm Diode	1.14×10^8	30.1	0.31×10^4	6.34
GROUP-3 980nm Diode	1.10×10^8	28.3	0.26×10^4	4.83
GROUP-4 Nd:YAG	1.07×10^8	32.2	0.21×10^4	3.33

[Table/Fig-1]: Shows the CFU/ml of all groups of pre treatment and post disinfection after 24 h

wavelength of 810 nm and 980 nm, with a pulse duration of 100 µs. For all samples the total irradiation time was 10 sec. The laser radiation was delivered into the root canals via a flexible quartz glass fiber with a core diameter of 200 µm. The laser application was performed with the fibre optic cable in circular and forwardbackward movements in contact with the root canal wall.

Determination of the Number of Bacteria

Each root canal was then rinsed with 1 ml of sterile BHI broth which was collected. Broth was plated by spread plate method on Brain Heart Infusion Agar medium, and incubated at a temperature of 37 °C overnight. The plates were incubated for 24 h at 37°C and colonies of *E. faecalis* were counted using a stereomicroscope (Zeiss, Oberkochen, Germany) and recorded as number of Colony Forming Unit (CFU) / ml.

RESULTS

The bacterial counts before the radiation in the control group and those after therapy in the four experimental groups are presented in [Table/Fig-1]. There was a statistically significant reduction in the bacterial count between the laser groups. [Table/Fig-1] shows the CFU/ml of all groups of pre treatment and post disinfection after 24 h. All specimens of the four groups showed bacterial growth and there was no statistically significant difference between the four groups. After 24 h, a statistically significant difference in bacterial reduction was seen with group-4 (Nd:YAG laser) when compared to group-1($Ca(OH0)_2$.

DISCUSSION

In order to achieve better results of endodontic treatment, a great deal of effort has been made to find another approach. Antibacterial effect of dental laser is based upon that thermal mechanism. Reduction or even complete elimination of bacteria has been achieved by several laser systems. Sodium hypochlorite (NaOCl) is known as a strong antibacterial agent and has been used in 0.5-5.25% concentrations in endodontic practices for many years.

The effect of NaOCl was well known, most of the organisms present inside the dentinal tubules, and lateral canals. The presence of a smear layer after instrumentation reduces the effectiveness of irrigants and temporary dressings in disinfecting dentinal tubules. EDTA has the effect of removing smear layers,which helps the disinfection process in root canals [5,6].

Research indicates that, besides improving the treatment of dental hard tissue and forming the root canal by using laser radiation, the ancillary effects of laser light on endodontic bacteria are postulated. The laser radiation may be transmitted through quartz optical fibers, a property that could facilitate introducing laser light around canal curvatures and irregularities. Finding a method to provide disinfection in root canals without causing a cytotoxic effect on peripheral tissue is necessary. In this study, canals contaminated with *E. faecalis* and irradiated using fiber optic directed light from a pulsed Nd:YAG laser and Diode laser [1,4,5,6].

In this study, the role of Nd:YAG and Diode lasers in root canal disinfection was defined using bacteriological testing. *E.faecalis*, which are well-known endodontic pathogen, were selected for the infection of the root canals. *E. faecalis* is a particular clinical relevance because these species have frequently been associated with therapy-resistant infections.

It is known that the antibacterial activity of $Ca(OH)_2$ depends on its high pH. The alkalinity of this agent destroys bacterial cell membranes and protein structures (Siqueira & de uzeda 1996). However, the initial high pH of $Ca(OH)_2$ at 12.3 would reduced to a pH of 10.3 when it was placed into the root canals.

This pH reduction is due to because of buffering effect of the radicular dentine. It has been known that the *E. faecalis* can survive at a pH as high as 11.5 Evans et al., hence, with the lower pH value of Ca(OH)2, the *E. faecalis* in the dentinal tubules could not be removed effectively [1,2].

This study showed that both lasers were found to be more effective than the $Ca(OH)_2$ in the infected tooth section models. Although group-4 (Nd:YAG) showed the most effective antimicrobial activity among the other test groups.

GUTKNECHT et al., achieved an average of 99.92 % bacterial reduction in the root canal using the Nd:YAG laser with standard settings of 15 Hz at 100 mJ = 1.5 W, repeated four times for 5 to 8 sec [7,8].

ROONEY et al., and HARDEE et al., described Bacterial reduction of 99 %, when using a Nd:YAG laser in different experimental designs and bacterial combinations. KLINKE et al., were able to prove a bactericidal effect of the Nd:YAG laser at a depth of 1,000 µm. In comparison, a rinsing solution, such as NaOCl, only achieves effective bacterial reduction up to a depth of 100 µm [9,10].

Nd:YAG lasers show the best results in transmission and microorganism reduction measurements. Even at penetration depths exceeding 1.000 µm, 85 % reduction is achieved. The 810 nm diode laser is the second-choice laser source. Micro-biological studies have shown that this source provides the second highest micro-organism reduction, approximately 63%. This is nevertheless significantly lower than with Nd:YAG lasers. 980 nm diode lasers may also be an option although high transmission is achieved due to its higher absorption in water [9,10].

LIMITATIONS OF THE STUDY

It is very important for clinicians to understand the type of clinical methods that appear in the literature and the inherent strengths and limitations of each study. The three possible alternative explanations, wavelength, cycles, and time, must be considered for laser studies. The operator should be well trained to use a laser device. All lasers used at wrong parameters and time can cause damage to root canal walls. Finally, cost of the equipment is too high.

CONCLUSION

From the results of this study it could be concluded that Nd:YAG laser was the most effective to eliminate and disinfection of root canals with *E. faecalis*. 980 diode laser was significantly more effective than 810nm diode laser. That all the wavelengths most commonly used in today's dentistry are also suitable for the disinfection of root canals. The choice of laser, proper wave length and parameters are important for a safe and success of the root canal therapy.

REFERENCES

[1] Mohammed Ahmeduddin, Nagesh B, Narasimha Reddy K, Sharath Raj K An assessment of bactericidal effect of two different types of lasers on *Enterococcus faecalis*: An in vitro study. *J Dent Lasers.* 2012; 6: 2-6.

[2] Manikandan R, Mithra N Hegde Comparative evaluation of biofilm formation ability of *E.faecalis* in alkaline conditions and its susceptibility to endodontic irrigant regimens – An In vitro microbiological study. *Journal of Dental and Medical Sciences.* (IOSR-JDMS). 2013; vol 4:49-52.3.

[3] Stuart CH, Schwartz SA. *Enterococcus faecalis*; Its role In root canal treatment failure and current concepts in retreatment. *J Endod.* 2006; 32: 93-98.

[4] Ambica Khetarpal, Ramanathan Ravi. Successful Endodontic Management Using Er,Cr:YSGG Laser Disinfection of Root Canal in a Case of Large Periapical Pathology. *International Journal of Dental Sciences and Research.* 2013; Vol 1, No. 3: 63-66.
[5] Gerek M, Asci S. Ex Vivo evaluation of antibacterial effects of ND:YAG and Diode lasers in root canals. *Biotechnol. & Biotechnol. Eq*. 2010; 2031-2034.
[6] Ali Safan, Tareq Youssef. Antibacterial Effect of Silver & Gold Nanoparticles and Diode Laser against Lactobacillus acidophilus bacteria. *International Journal of Advanced Research.* 2014; Volume 2, Issue 8: 34-38.
[7] Norbert. Gutknecht State of the Art in Lasers for Dentistry. *Journal of the Laser and Health. Academy.* 2008; 1.
[8] Norbert Gutknecht. Lasers in Endodontics. *Journal of the Laser and Health Academy*. 2008; 2.
[9] WL CHAi. Evaluation of Antimicrobial Efficacy of Antibiotics and Calcium Hydroxide against *Enterococcus faecalis* Biofilm in Dentine. *Sains Malaysiana.* 2013; 42(1):73–80.
[10] Kimura Y, Wilder-Smith P, Matsumoto K. Lasers in endodontics: a review. *Int Endod J.* 2000;33(3):173-85.

PARTICULARS OF CONTRIBUTORS:
1. Reader, Department of Conservative Dentistry and Endodontics, Sibar Dental College, Guntur, Andhra Pradesh, India.
2. Senior Lecturer, Department of Conservative Dentistry and Endodontics, Sibar Dental College, Guntur, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Narasimha Reddy Kanumuru,
Reader, Sibar Dental College, Guntur, Andhra Pradesh-522403, India.
Phone : 9160247894, E-mail : endonarsi@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 05, 2014**
Date of Peer Review: **Jul 01, 2014**
Date of Acceptance: **Jul 05, 2014**
Date of Publishing: **Nov 20, 2014**