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Comparative Evaluation of Antimicrobial Efficiency Of Diode Laser, Sodium Hypochlorite And Their Synergistic Effect Against *Enterococccus faecalis* Contaminated Root Canals-An *in vitro* Study

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ABSTRACT

Aim: To compare the efficacy of Diode laser irradiation procedure with that of sodium hypochlorite irrigation procedure and their synergistic effect against *Enterococcus faecalis*- contaminated root canals.
Materials and methods: Forty root canals of extracted central incisor teeth with straight roots were chosen. The canals were enlarged with 40 size K file and randomly divided into four groups of 10 teeth each. After sterilization, all roots will be inoculated with *Enterococcus faecalis* for 7 days at 37˚C. The first group was used as a control, the second group was irrigated with 5.25% Naocl solution for 5 min, third group was irradiated with 810-nm Diode laser at output power from 2 W for 5*5secs and fourth group was irrigated with 5.25% Naocl followed by irradiation with Diode laser after drying the canal. The disinfecting efficacy of the groups were tested by collecting dentine chips from the inner walls of the canal and counting colony forming units of viable *E. faecalis* on agar plates. Statistical analysis were done using Anova and Post Hoc tukey test.
Results: Diode laser alone and Diode laser with sodium hypochlorite shows complete elimination of *E.faecalis* from the root canal.

Key words: Diode laser, *Enterococcus faecalis*.

Introduction

The outcome of root canal treatment is based on efficient disinfection of the root canal system and prevention of reinfection[1]. In other words, according to the role of micro-organisms in apical periodontitis, endodontic treatment should focus on removal of microbial colonization from root canal system (through antiseptic procedures) and preventing the entry of new micro-organisms to the root canals (by aseptic techniques) [2]. After pulpal infection, bacteria can penetrate into the dentinal tubules as well as periapical tissue.

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Use of mechanical instrumentation alone cannot sufficiently debride and clean this complete tubular network.[3,4]. Many irrigation solutions have been used for root canal treatment along with mechanical instrumentation to achieve better debridement.They do not achieve a complete disinfection of root canal space mainly in the apical third and the inner layers of dentin. [5].During root canal infection, the microenvironment of root canal favors the selection of few bacterial species like *Enterococcus faecalis, Streptococcus anginosus and Fusobacteriumnucleatum*. [6] *E.faecalis*is a non fastidious, therapy resistant, gram positive facultative anaerobe that can proficiently invade dentinal tubules, survive chemomechanical instrumentation and intracanal medication, adapt to altered nutrient supply and continue to remain viable within the dentinal tubules[7]. Combination therapy using various medicaments or irrigation solutions together or in succession has been reported to be more effective for canal disinfection. The use of MTAD (a mixture of a tetracycline isomer, an

acid and a detergent) as a final rinse after using 1.3% NaOCl during instrumentation has been advocated to be effective against *E. faecalis*[8]. Furthermore, laser-assisted endodontics using high-power diode laser irradiation followed by canal irrigation with 0.5% NaOCl and 17% EDTA-T is able to eliminate *E. faecalis* completely [9].The use of lasers like high power Diode laser in endodontics is an innovative approach for disinfection, providing access to formerly unreachable parts of the tubular network, due to their ability to penetrate dental tissues better than irrigant solutions[9-11].In this *in vitro* study, we investigated the application of diode laser irradiation alone and after use of disinfecting irrigation solutions in *E. faecalis*-contaminated root canals. The hypothesis tested was that laser irradiation and their combination with irrigation solutions would eliminate E.*faecalis* completely from the root canal.

Materials and methods

The study sample consisted of 40 extracted human maxillary incisors. All teeth were extracted because of periodontal disease . All teeth had completely developed roots and were without root caries or previous endodontic treatment. The presence of a single canal was determined by radiographs taken in both mesiodistal andbuccolingual directions.and stored in 0.9% physiological saline. The teeth were decoronated using water cooled diamond disk and roots standardized to a length of 15 mm. A 10 K file was introduced into each canal until it appeared at the apical foramen;and, working length was established by subtracting 0.5 mm from this length. Apical third of roots were cleaned and shaped to 40 size K file using step back technique for standardization. During instrumentation, the root canals were irrigated with 3 ml of 1% NaOCl at each change of file followed by 17% EDTA solution for 1 minute for smear layer removal; and, later rinsed with saline and dried using paper points.The apical foramen was sealed using light-cured restorative glass ionomer cement. The other surfaces of the roots were covered with two layers of nail varnish.All the teeth were sterlized in autoclave under 15 psi pressure at 121˚c for 30 mins.

Preparation of inoculation and contamination of root canals

An overnight pure culture of *E. faecalis* (ATCC 29212) in Brain Heart Infusion (BHI) broth at a concentration of 1.5 × 108 CFU/ml was used for inoculation. The bacterial suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale. A 0.01-ml aliquot of the suspension was inoculated into each canal using a sterile insulin syringe except 5 teeth which served as negative control. Then the samples were incubated for two weeks under aerobic conditions at 37ºC. The inoculum inside the canal was replaced with 0.01 ml of fresh bacterial suspension every other day for seven days. After incubation, the contaminated roots were randomly divided into 4 groups.

Grouping:

Group I Control

Group Ia (positive control): 5 teeth were irrigated with 1ml of saline solution.

Group Ib(negative control): 5 teeth served as negative control without any irrigation.

Group II (5.25%sodium hypochlorite)

10 teeth were irrigated with 5.25% of sodium hypochlorite and left in canal for 5minutes.

Group III (Diode laser)

10 teeth were irradiated with laser irradiation was performed 5 times for 5 s each time, with a 15-s interval between irradiations. Laser treatments were carried out with a diode laser, at a wavelength of 810 nm and output power of 2 W with the repeated pulse mode, using a pulse duration of 20 ms and a pulse interval of 40 ms. The laser irradiation was delivered into the canal up to 1 mm short of the working length via a fiber tip 400 μm in diameter.

Group IV (combination of 5.25% Naocl and diode laser)

10 teeth were irrigated with 5.25% of sodium hypochlorite and left in canal for 5 minutes after irrigation canals were dried with paper points and subsequently laser irradiation done as group III.

Irradiation was performed with circling movements from the apical part toward the coronal part (step-back technique) without any water spray or air cooling. After irrigation canals were filled with normal saline as a transfer fluid. Sampling from inside the canals was done using a sterile #25 H-file, and circumferential filing was performed for 20 seconds to collect dentin chips, mostly from the coronal and mid parts of the canal. A sterile #35 K-file was used for sampling from the apical part by reaming for 20seconds. Sterile paper points were used to collect the transfer fluid and dentin chips. Sterile paper

points and sampling H- and K-files had been placed into a test tube containing10 ml of sterile saline and vortexed for 20 seconds. Fifty microliters of the vortexed saline was applied to Tryptic soy agar and incubated at 37ºC for 48 h. All procedures were carried out under sterile and aseptic conditions. The CFU/ml for each plate was calculated using a bacterial colony counter (Table 1). The mean and standard deviation of CFU values were calculated for the samples. Degrees of disinfection in the experimental subgroups were calculated in relation to the controls. The CFU values were analyzed by Anova and Post Hoc tukey test (Table 2 and Table 3).

Results

The mean CFU for all groups were: Group 1a = 295 x 102, Group 1b=0 ,Group 2 = 39 x 102, Group 3 = 0, Group 4 = 0 CFU/mL. When comparing groups,Group 1shows high statistically significant difference to all other groups. Group 2 shows *E.faecalis* colony but it does not shows statistically significant difference Group3, Group 4. Whereas, Group 3,Group 4 shows complete eradication of E.*faealis.*

Discussion

The primary objective of endodontic treatment is the disinfection of the root canal, and its three dimensional network of dentinal tubules[11,12]. *E. faecalis*, as a biofilm-forming pathogen, was chosen for this investigation since it has been used for evaluation of the antibactericidal effects of several irrigation solutions and various laser devices [13-16],Itis a non-fastidious, therapy resistant, gram positive facultative anaerobe that can proficiently invade dentinal tubules, survive chemomechanical instrumentation and intracanal medication, adapt to altered nutrient supply and continue to remain viable within the dentinal tubule [5,7]. Final rinse of 5.25% NaOCl increases the bactericidal effect in dentinal tubules. The bactericidal effect of NaOCl is due to the effect of hypochlorous acid and active chlorine which exerts antimicrobial effect[17,18]. EDTA used in irrigation dissolves the inorganic smear layer and increases the permeability of dentinal tubules[18].However, despite this superior disinfection protocol,previous studies show depth of penetration of irrigants to be limited to 100 μm, Whereas, *E. faecalis*is known to penetrate to a depth of 600-1000 μm.[6,19].This study and the previous ones[20] have demonstrated that disinfection of root dentin is not achieved by chemomechanical preparation alone. Bacteria deep in dentinal tubules are apparently protected from instrumentation and irrigation, making their removal or eradication difficult[20].When 908 nm diode laser was used alone or in conjunction with NaOCl shows a complete elimination of *E.faecalis*. The superior bactericidal effect of diode laser irradiation could be attributed to its greater depth of penetration (up to 1000 μm into dentinal tubules) when compared to the penetration power of chemical disinfectants, which is limited to 100 μm[6,19].Laser irradiation with its inherent properties of light scattering, local intensity enhancement and attenuation allows light penetration deeper in the dentin tubules contributing to a superior antimicrobial efficacy[9,11,17].The diode laser causes a thermal photodisruptive action in the unreachable parts of dentin, resulting in an enhanced bactericidal effect in the root canal dentin[19].These results were congruent with the study by Eliana Barbaosa [9], Senemselvikuvettli[21],Thomas preethee[22], where NaOCl/EDTA irrigation followed by diode laser irradiation demonstrated complete disinfection compared to the disinfectant treated group alone. Contrary to the results of our study, D. Jha *et al*. demonstrated the inability of laser and rotary instrumentation to bring about the complete disinfection of root canals. The less favourable results could be possibly due to lesser disinfecting capability of the Erbium, Chromium doped Yttrium Scandium Gallium Garnet (Er, Cr: YSGG) laser set at a lower power (1.5W); and, that the infected teeth were laser instrumented first followed by irrigation[23].

Conclusion

On the basis of present study it can be concluded that Diode laser alone /Diode laser with NaOCl completely eradicated E.*faecalis* from the root canal. Due to limitations and complications during treatment procedures in a clinical situation, further studies are required to investigate the clinical effectiveness of this approach.

Table 1: Colony forming units of *E. Faecalis* of varying groups

Control (CFU)		Naocl (CFU)	Laser (CFU)	Naocl+Laser (CFU)
300	0	42	0	0
280	0	0	0	0
274	0	0	0	0
330	0	64	0	0
293	0	0	0	0
		0	0	0
		96	0	0
		76	0	0
		0	0	0
		114	0	0

Table 2: One way Analysis of Variance test for demonstrating differences in number of *Enterococcus faecalis* colony forming units/mg following laser and chemical disinfection in different groups in the study

Group	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	ANOVA Test Value – F	P Value
Control positive	295.40	21.90434	9.7959	268.20	322.5978	156.161	.000***
Control negative	.0000	.00000	.00000	.0000	.0000		
NaOCl	39.200	45.31568	14.330	6.7831	71.6169		
Laser	.0000	.00000	.00000	.0000	.0000		
Laser with NaOCl	.0000	.00000	.00000	.0000	.0000		

Table 3: Post Hoc tukey values

Multiple Comparisons				
(I) Groups	(J) Groups			
		Mean Difference (I-J)	Std. Error	Sig.
Control positive	Control negative	295.40000*	15.27066	.000
	NaOCl	256.20000*	13.22478	.000
	Laser	295.20000*	13.22478	.000
	Laser with NaOCl	295.40000*	13.22478	.000
Control negative	Control positive	-295.40000*	15.27066	.000

	NaOCl	-39.20000	13.22478	.054
	Laser	-.20000	13.22478	1.000
	Laser with NaOCl	.00000	13.22478	1.000
NaOCl	Control positive	-256.20000*	13.22478	.000
	Control negative	39.20000	13.22478	.054
	Laser	39.00000*	10.79799	.009
	Laser with NaOCl	39.20000*	10.79799	.009
Laser	Control positive	-295.20000*	13.22478	.000
	Control negative	.20000	13.22478	1.000
	NaOCl	-39.00000*	10.79799	.009
	Laser with NaOCl	.20000	10.79799	1.000
Laser with NaOCl	Control positive	-295.40000*	13.22478	.000
	Control negative	.00000	13.22478	1.000
	NaOCl	-39.20000*	10.79799	.009
	Laser	-.20000	10.79799	1.000

***. The mean difference is significant at the 0.05 level.**

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