

Comparative Evaluation of Efficacy of Nd: YAG Laser and 5.25 % Sodium Hypochlorite with and without an Antifungal Agent on *Candida Albicans*

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ABSTRACT

Background: The microflora in failed endodontic treatment consist mainly of, *Enterococcus faecalis*, *Candida albicans* and *Actinomyces concommitans*. Evaluating a protocol for disinfection in canals without having a toxic effect on peripheral tissue is important. The aim of this in vitro study was to check the efficacy of Nd: YAG Laser and 5.25 % Sodium Hypochlorite with and without an Antifungal Agent.

Materials and Methods: Root canals of single rooted permanent teeth were enlarged using the Protaper nickel titanium rotary system up to size 40 at 1 mm from the apical foramen. The inoculation of canals of the experimental teeth were done with 0.5ml of freshly prepared suspension and stored. Then the viles were incubated at 91% humidity and 37 degree Celsius for 96 hours. After 24 hours the experimental teeth were replaced with immediately prepared suspension containing *Candida albicans*. At 48 hours samples were plated on 4% SD agar plate to check the growth of *Candida albicans*. The samples were then divided into four groups- Group I- irrigation with sodium hypochlorite, Group II- Irrigation with sodium hypochlorite and antifungal agent, Group III- disinfection with LASER, Group IV- disinfection with LASER and antifungal agent.

Conclusion-Under the limitations of a laboratory study of this kind, it can be concluded that Clotrimazole had a synergistic effect when used in conjunction with different disinfection protocols.

Keywords: Antifungal efficacy, 5.25% sodium hypochlorite, *Candida albicans*.

INTRODUCTION

The key to a successful root canal treatment is complete disinfection. The presence of bacteria within the root canal system is main reason for

failure.¹ Anaerobic species are most predominant among the various microflora present within the root canal system.² Kakehashi et al¹ proved that bacteria are the most common cause for pulpal

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disease; many studies have revealed even fungi and recently viruses also in the incidence of endodontic infections. The microflora in failed endodontic treatment consist mainly of, *Enterococcus faecalis*, *Actinomyces concomitans*, and *Candida albicans*.^{1,2}

In the normal microbial flora of the oral cavity, fungi is the opportunistic pathogens seen in 25% of healthy adults and 50% of hospitalized patients.³ The genus *Candida* with *C. albicans* is most predominant and isolated yeast from the oral cavity followed by *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida kefyr*.³ The incidence of *Candida* from infected root canals to range from 1% to 17% since last 10 years.^{14,15,16} Antifungal treatment of infected root canals is evident. No specific antifungal agent is used either as an intracanal medicament or for irrigation in the infected root canals. The NaOCl is a commonly used irrigant, but some microorganisms survive inside dentinal tubules or other inaccessible areas.⁷ Clotrimazole, is an antifungal drug belongs to a family of Azoles and is useful in treating systemic mycoses. It is available in different forms such as tablets, pastes, gels, creams, powders, and topical solutions. The composition of the solution is 1% Clotrimazole in a base of Propylene Glycol and Glycerine¹⁰. So it is necessary to find a method to provide disinfection in root canals without causing a cytotoxic effect on peripheral tissue is necessary.

MATERIALS AND METHOD

Fifty extracted human permanent single rooted teeth were then decoronated at the Cementoenamel junction after taking radiograph to confirm presence of single canal and pulpal remnants were extirpated. Canals were widened using the protaper nickel titanium rotary system up to size 30 at 1 mm from apex. Apical foramina were sealed with GIC type II. Physiologic saline irrigation was performed using endodontic needle to irrigate the canal. Subsequently the roots were sterilized.

Reference strains of *Candida albicans* obtained from National Chemical Laboratory (ATCC 10231). The inoculation of canals of the experimental teeth were done with 0.5ml of freshly prepared suspension and stored. Then the vials were incubated at 91% humidity and 37 degree Celsius for 96 hours. After 24 hours the experimental teeth were replaced with

immediately prepared suspension containing *Candida albicans*. At 48 hours samples were plated on 4% SD agar plate to check the growth of *Candida albicans*. At the end of 96 hrs excess fluid in canal was removed and teeth were subjected to experimental groups. Teeth were divided into five groups randomly in which each comprised of 10 teeth. The time of contact of each irrigant for group I, II, IV and V was 1 min. for 2ml of solution and a final irrigation of 5 ml of distilled water was given. Control group was irrigated with normal saline.

The Nd: YAG laser device of wavelength of 1064 nm was used for group III and IV. For laser disinfection of canal the fiber was put at a distance of 1 mm from the apex and moved in three repeated cycles at 10 second applications and then 10 seconds. All the experimental groups were then irrigated with 20 ml of saline, and then using sterile absorbent paper points canals were dried. Samples were removed and was plated on dextrose agar. The plates were then incubated at 91% humidity and 36 degree Celsius for 48 hrs. After the incubation period, the number of Colony Forming Unit per ml was counted and the growth of *Candida albicans* was evaluated. The number of Colony Forming Unit Colony Forming Unit of candid served as a gold standard of antifungal activity of the samples. Mean and Standard deviation was calculated, data was analyzed by using ANOVA test, followed by Tukey post hoc and the student's "t" test.

RESULTS

When 1% Clotrimazole was included along with Nd: YAG laser and Hypochlorite, there was statistically significant difference in the Colony forming units. ($P < 0.001$) (Figure 1).

DISCUSSION

The study was conducted to evaluate the efficacy of Nd: YAG Laser and 5.25 % Sodium Hypochlorite with and without an Antifungal Agent on *Candida albicans*. The effectiveness of NaOCl is well documented in literature¹¹. When smear layer is absent NaOCl displayed antifungal activity after 30 minutes. According to results obtained in our study Nd: YAG laser when used in conjunction with an antifungal agent (1% Clotrimazole) was more effective than when used alone and NaOCl used with and without an antifungal agent.

Table 1: Shows the mean growth of CFUs of *Candida albicans*. Among all the disinfection protocols evaluated, Nd:YAG laser with an antifungal agent was the most effective and statistically significant when compared to other groups.

TABLE 1: The Mean Growth of CFUs of <i>Candida Albicans</i> 1x10 ³ ml				
Group I (Hypochlorite)	Group II (Hypochlorite + Antifungal Agent)	Group III (Nd:YAG Laser)	Group IV (Nd:YAG Laser + Antifungal Agent)	Control Group
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
27.68±1.27	6.61±0.65	22.86±1.05	4.22±0.74	212±16.12

Table 2: CFU count of *Candida Albicans* 1*10³ ml for all five groups.

Groups Sr No (Samples)	Hypochlorite I	Hypochlorite + Antifungal Agent II	Nd:YAG laser III	Nd:YAG laser + Antifungal Agent IV	Control Group V
CFU count of <i>Candida Albicans</i> 1*10 ³ ml					
1	28.8	6.8	21.6	4.0	200
2	26.2	7.2	21.5	3.8	225
3	27.4	6.6	24.2	2.9	222
4	25.5	6.2	23.2	4.4	240
5	28.6	5.8	22.9	5.1	210
6	26.6	6.6	21.7	4.8	190
7	27.5	6.0	23.0	3.7	188
8	29.5	5.9	24.5	4.2	215
9	28.5	7.2	23.5	3.9	220
10	28.2	7.8	22.5	5.4	210

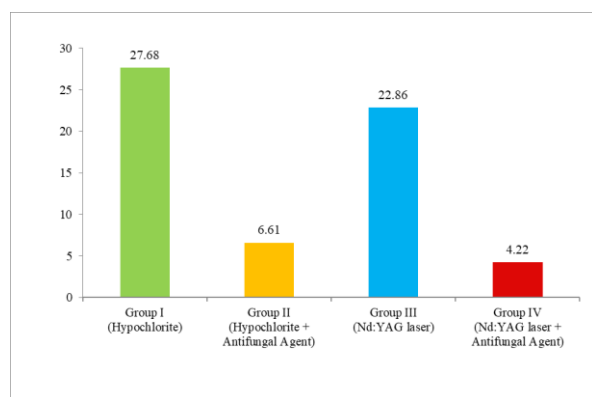


Fig 1: Distribution of mean values of CFU of *Candida albicans* in Group I, Group II, Group III, and Group IV.

Sodium hypochlorite on contact with tissue proteins, nitrogen, formaldehyde, and acetaldehyde are formed within a short time and peptide links are broken, resulting in dissolution of the proteins.

5.25% NaOCl completely destroyed *C. albicans*, *E. faecalis*, and *Bacillus* species.¹² The efficacy of NaOCl is due to its ability to oxidize and hydrolyze cell proteins and to osmotically draw fluids out of cells due to its hypertonicity.¹³ Research indicates that, laser light has an ancillary effect on endodontic bacteria^{8,9}. The disinfection results from converting radiant energy into thermal energy inside tissues.¹⁴ Studies have documented the bactericidal effects of Nd:YAG Laser.^{8,15} Consequently, it is efficient for root canal disinfection at energy levels of 1.5W and 30 MJ and is recommended for root canal disinfection.⁸ Schoop et al.¹⁵ reported that Nd: YAG and Diode Lasers at 1W are effective for *E. faecalis* and *Candida albicans*. In this study, irradiation with the Nd: YAG Laser was applied with a constant repetition rate of 15 Hz and output of 1.5W, with the Diode Laser was applied at 2W. Clotrimazole is a substituted imidazole, it is a broad-spectrum antifungal activity covering the *Candida* species,

dermatophytes, and some gram-positive anaerobic bacteria such as *Staphylococcus aureus*¹⁸. Hence, the use of 1% Clotrimazole can be recommended as an adjunct in the final irrigation protocol. The results of this study were highly significant differences between the four disinfection groups when evaluated for their anti-fungal activity against *Candida albicans*. According to the results obtained in this study, Nd: YAG Laser when used alone was much more effective than Sodium hypochlorite used alone.

This can be attributed to the fact that Nd:YAG laser has the ability to disinfect the root canal principally by heating effect. Nd:YAG laser has been shown to be useful for the removal of the smear layer and debris^{16,17} which might have increased the effect of laser and the antifungal agent. Also Clotrimazole causes the disruption in membrane permeability. The synergistic effect of laser and antifungal agent resulted in marked reduction in the CFUs of *Candida albicans*. Hence, suggesting that Clotrimazole is used as a root canal irrigation, to increase the antifungal efficacy.

CONCLUSION

Under the limitations of a laboratory study of this kind, it can be concluded that Clotrimazole had a synergistic effect when used in conjunction with different disinfection protocols. Further clinical evaluation is necessary to come to a better conclusion regarding the antifungal efficacy of various disinfection systems, with and without the addition of antifungal agents.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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