Original Article

Is Nd:YAG laser effective for inhibiting the growth of *Candida albicans* and *Candida tropicalis*?

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Abstract

Objective: *Candida albicans* and *Candida tropicalis* are the most common fungal species in humans. The present study aimed to investigate the effect of neodymium-doped yttrium aluminum garnet (Nd:YAG) laser on inhibiting the growth of *Candida albicans* and *Candida tropicalis* in vitro.

Methods: *Candida albicans* and *Candida tropicalis* species were cultured in sub-dextrose agar containing chloramphenicol and exposed to Nd:YAG laser (1064 nm). The laser was emitted at the pulse frequency of 1 Hz (1 pulse per second) for 7 or 13 seconds. At each pulse duration, the energies of 40, 60, 80, or 100 mJ were delivered to microbial plates. After radiation, the number of colonies was counted and reported as colony-forming units per milliliter (CFU/mL).

Results: There was a significant reduction in the number of *Candida albicans* and *Candida tropicalis* colonies after Nd:YAG laser radiation, compared to the control group ($P<0.05$). At the pulse duration of 7 seconds, there was a significant difference in the number of *Candida albicans* colonies between the pulse energy of 40 mJ with other pulse energies ($P<0.05$). At the pulse duration of 13 seconds, the energies of 80 mJ and 100 mJ were significantly more potent at killing *Candida tropicalis* than other pulse energies ($P<0.05$). Increasing the duration of irradiation from 7 to 13 seconds was effective at killing *Candida* species at most pulse energies ($P<0.05$).

Conclusions: Nd:YAG laser is effective in inhibiting the growth of *Candida* species. Under the conditions of this study, the antifungal effect of Nd:YAG laser improved with increasing pulse energy and duration of laser irradiation.
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Keywords: *Antifungal, Candida albicans, Candida tropicalis, Nd:YAG laser*

Introduction

Candida species are coexisting microorganisms in balance with the human body and coexist in 75% of healthy individuals (1). Most people control the growth and spread of these opportunistic fungi through physiological defense mechanisms and healthy immune systems. However, under certain conditions and in the presence of predisposing factors such as diabetes, immune deficiency, and the use of widespread antibiotics, there is an opportunity for *Candida* species to become pathogenic and coexist with the possibility of developing candidiasis. This disease is the most common opportunistic fungal infection in the host (2, 3).

Among the species of *Candida* pathogen, *Candida albicans* is the most pathogenic and the most common fungal infection in humans, followed by *Candida tropicalis* (2, 4). Today, the presence of *Candida albicans* in dental caries has been verified, and the relationship between *Candida albicans* and dentin caries has been discussed (5, 6). Dental prostheses support the growth of *Candida* species and may provide the basis for oral lesions. *Candida albicans* and *Candida tropicalis* appear in these microbial colonies and are resistant to conventional treatments, implying the need to search for new modalities (7). Extensive use of systemic and topical antifungals for treating oral candidiasis has led to the resistance of *Candida albicans* to these substances (8).

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The invention of the laser by Miaman in 1960 (9) led to the development of various applications of this device in dental practice. The effects of lasers in dental structures depend on the laser wavelength, duration of irradiation, pulse energy, pulse length, power of the device, and depth of penetration into the tissue (10, 11). Lasers have potential benefits such as antibacterial and antifungal effects, detoxification, and removal of the epithelial lining and granulation tissue, which are desirable in surgical and periodontal treatments (8, 12, 13).

The neodymium-doped yttrium aluminum garnet (Nd: YAG) laser emits a wavelength of 1064 nm and is in the near-infrared range of the electromagnetic spectrum (14). The Nd: YAG laser has been used in various periodontal treatments, including soft tissue surgery and curettage (15, 16). The antibacterial effects of Nd:YAG laser and its efficacy in reducing endodontic biofilms and periodontal inflammation has been previously demonstrated (17, 18). There is limited information about the efficacy of Nd: YAG laser in eradicating fungal microorganisms (19). Therefore, the present study was conducted to assess the effect of Nd:YAG laser on the viability of two *Candida* species including *Candida albicans* and *Candida tropicalis.*

Materials and Methods

This research was approved by the Ethical Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IRAJUMS.REC.1399.790).

The *Candida* species were prepared from the Pasteur Institute of Iran (*Candida albicans*: 5027PTCC; *Candida tropicalis*: 1643PTCC). The equal volumes of microorganisms were transferred to the sub-dextrose agar containing chloramphenicol and incubated at 37˚C for 24 h. After that, the concentrations of 1.5×10^8 CFU /ml of microorganisms were prepared. Nine groups were evaluated in this study consisting of 4 *Candida albicans* groups, 4 *Candida tropicalis* groups, and one control group. The experiments were performed 5 times for each group.

The nanosecond pulse Nd:YAG laser device (SA Iran Electro-Optical Industries) was used in the present investigation. This laser emitted short pulses with a duration of 10 nanoseconds (ns), wavelength of 1064 nm, and a maximum energy of 100 mJ per pulse. The laser radiation in this study was performed with various pulse energies at an 8 cm distance from the microbial plate using the pulse frequency of 1 Hz (1 pulse per second) for 7 or 13 seconds (equal to 7 or 13 pulse numbers). At each pulse duration, the energies of 40, 60, 80, or 100 mJ were delivered to the plates.

Following radiation, the volume content of 10 µl was transferred from each well to the Mueller Hinton agar. After 24 hours of incubation, the number of colonies was counted and reported as colony-forming units per milliliter (CFU/mL). No intervention was performed in the control group.

Statistical analysis

The Shapiro-Wilk test confirmed the normality of the data distribution (P>0.05). One-way analysis of variance was run to detect any significant difference in colony counts at each duration (7 or 13 seconds), followed by Bonferroni's posthoc test for pairwise comparisons. A P-value<0.05 was considered statistically significant.

Results

The effect of Nd:YAG laser on growth inhibition of Candida albicans

ANOVA revealed a significant difference in the counts of *Candida albicans* among the study groups at both pulse durations (P<0.05; Table 1). Pairwise comparisons demonstrated a significant difference between all radiation doses and the control group either with a duration of 7 or 13 seconds (P<0.05; Table 1). At the pulse duration of 7 seconds, there was a significant difference between the energy of 40 mJ with other pulse energies (P<0.05; Table 1). However, there was no significant difference between various pulse energies in killing *Candida albicans* at the pulse duration of 13 seconds (P>0.05; Table 1).

Increasing the duration of irradiation from 7 to 13 seconds caused a significant reduction in the number of colonies at all energy pulses (P<0.05; Table 1) except for the pulse energy of 60 mJ where the reduction in the number of colonies was not significant (P=0.14; Table 1).

The effect of Nd:YAG laser on growth inhibition of Candida tropicalis

ANOVA indicated a significant difference in the counts of *Candida tropicalis* among the study groups at both pulse durations (P<0.05; Table 1). Pairwise comparisons exhibited a significant difference between all radiation doses and the control group following 7 or 13 seconds of Nd:YAG laser irradiation (P<0.05; Table 2). There was no significant difference between various pulse energies at 7 seconds of radiation (P>0.05; Table 2). At the pulse duration of 13 seconds, the samples exposed to 80 or 100 mJ pulse energies exhibited a significantly lower number of colonies than those subjected to 40 or 60 mJ energies (P<0.05; Table 2)

Table 1. The number of *Candida albicans* colonies after Nd:YAG laser irradiation at different pulse energies and durations (7 or 13 seconds)

Duration of radiation	Pulse energy (mJ)					
	control	40	60	80	100	P-value
7 Seconds	293.3 ± 89.99[c]	140.3 ± 71.8[b]	60.8 ± 10[a]	59.7 ± 10.2[a]	58.3 ± 15.6[a]	0.005
13 Seconds	293.3 ± 89.99[b]	52.2 ± 16.6[a]	50.7 ± 11.8[a]	39 ± 13.1[a]	36.7 ± 18.8[a]	0.003
P-value		0.03	0.14	0.01	0.049	

*The groups that have been defined by different letters indicate statistically significant differences at P<0.05.

Table 2. The number of *Candida tropicalis* colonies after Nd:YAG laser irradiation at different pulse energies and durations (7 or 13 seconds)

Duration of radiation	Pulse energy (mJ)					
	control	40	60	80	100	P-value
7 Seconds	735 ± 204.1[b]	449.6 ± 93.7[a]	448.8 ± 78.3[a]	406 ± 67.1[a]	384.7 ± 29.5[a]	0.03
13 Seconds	735 ± 204.1[c]	382.7 ± 28.5[b]	340.5 ± 82.1[b]	200.7 ± 24[a]	191.3 ± 68.6[a]	<0.001
P-value		0.15	0.04	<0.001	0.001	

*The groups that have been defined by different letters indicate statistically significant differences at P<0.05.

Increasing the duration of irradiation from 7 to 13 seconds caused a significant reduction in the number of colonies at all energy pulses (P<0.05; Table 2) except for the pulse energy of 40 mJ, where the reduction was not significant (P=0.15; Table 2).

Discussion

This study investigated the effect of various parameters of a nanosecond pulsed Nd: YAG laser on the colony counts of *Candida albicans* and *Candida tropicalis* in vitro. *Candida albicans* and *Candida tropicalis* are the main fungal diseases that cause acute and chronic lesions in patients with weakened immune systems. However, there is some evidence showing that the use of lasers to treat these patients can be accompanied by favorable results (2, 5).

The results of this study revealed that Nd:YAG laser was indeed effective at killing *Candida* species and caused a significant reduction in the number of colonies at all energies and durations of irradiation, as compared to the control group. Generally, the antifungal effects of the Nd:YAG laser improved by increasing the pulse energy and duration of laser irradiation.

The antibacterial and antifungal effectiveness of different lasers has been demonstrated in previous studies (20-22), but there is limited research on the effect of Nd:YAG laser on changing macroscopic, microscopic, physiological, and biochemical properties of fungi. The outcomes of this study are in agreement with the results of Grzech-Lesniak et al. (23) who found that the exposure of *Streptococcus mutans* and *Candida albicans* to Nd: YAG laser significantly reduced cell number and metabolism in macroscopic and microscopic analyses. Baroni et al. (24) exhibited that the use of Q-switched Nd:YAG laser reduced the invasiveness of *Candida albicans* in skin mycosis, downregulated inflammatory responses, and improved cell protection and antimicrobial defense. In contrast, Kasic et al. (3) Indicated that erbium family lasers (Er:YAG and Er,Cr:YSGG) caused a significant reduction in the counts of *Enterococcus faecalis* and *Candida albicans* biofilms in root canals, but Nd:YAG laser irradiation was not effective for this application.

According to the outcomes of this study, it appears that there is a dose-dependent relationship, in which increasing the energy leads to an increase in the destructive power of the laser on fungi. However, the difference in the number of colonies was not significant between some energy pulses. At the pulse duration of 7 seconds, there was a significant difference between the pulse energy of 40 mJ with other pulse energies (60, 80, and 100 mJ) for *Candida albicans*, but the efficacy of all pulse energies was similar for *Candida tropicalis* samples. At the pulse duration of 13 seconds, the efficacy

of all pulse energies was comparable against *Candida albicans*, but the energies of 80 mJ and 100 mJ were significantly more potent at killing *Candida tropicalis* than other pulse energies.

The effect of increasing the duration of irradiation was significant at most pulse energies for both *Candida* species. Based on the outcomes of this study, the higher the duration of radiation to the *Candida albicans* and *Candida tropicalis*, the more fungal colonies are destroyed. The only exceptions were observed at the pulse energy of 60 mJ for *Candida albicans* and at the pulse energy of 40 mJ for *Candida tropicalis*, where the reduction in the number of colonies was not significant after increasing the duration of radiation. Several studies indicated better efficacy of high radiation doses for eradicating microorganisms (23, 25), but the use of very high laser energies can damage surrounding tissues in clinical conditions and should be avoided. Therefore, selecting the optimal laser settings to achieve the best results with the lowest possible energy density is of utmost importance. The outcomes of this study suggest that the growth of Candida species can be significantly reduced by the application of Nd:YAG laser. The antifungal effect of Nd:YAG laser improves with increasing pulse energy and duration of laser irradiation, although the effect of radiation duration appears more potent at killing *Candida* species.

This study was performed in laboratory conditions that differ from the complex oral environment. Further, in vivo studies are suggested to assess the efficacy of different settings of Nd:YAG laser on the growth of *Candida albicans* and *Candida tropicalis* at different oral lesions.

Conclusions

Nd:YAG laser is effective in inhibiting the growth of *Candida* species. The antifungal effect of Nd:YAG laser improves with increasing pulse energy and duration of laser irradiation, although the effect of radiation duration appears more potent at killing *Candida* species.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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