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In Vitro Effect of a High-Intensity Laser on *Candida albicans* Colony Count

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Abstract

Introduction: This study assessed the effect of a high-intensity laser on *Candida albicans* colony count.

Methods: This *in vitro*, experimental study was conducted on standard-strain *C. albicans* (ATCC18804). Seventy-two samples with two different concentrations of *C. albicans* (10^4 cells/mL and 10^6 cells/mL) were randomly assigned to three groups of control, laser and nystatin. Each group included 12 samples from each concentration. In the nystatin group, 10 cc of nystatin suspension was added to the samples and mixed for 30-60 seconds. In the laser group, the Ga-Al-Ar diode laser with a 940-nm wavelength and 2-W power was irradiated to the samples with 0.4 mm tip diameter in non-contact mode (1-mm distance) at a speed of 1 mm/s. The suspensions in the three groups were cultured on the Sabouraud dextrose agar culture medium and incubated at 37°C for 24 hours. The number of *C. albicans* colonies was then counted and reported. The three groups were compared by ANOVA and Tukey's test (alpha = 0.05).

Results: In both concentrations, the mean colony count in the nystatin group was significantly lower than that in the control ($P<0.05$) and laser ($P<0.05$) groups. The mean colony count in the laser group was significantly lower than that in the control group ($P<0.05$).

Conclusion: The high-intensity laser with a 940-nm wavelength and 2-W power in non-contact mode can significantly decrease the *C. albicans* colony count in vitro, but its effect is lower than that of nystatin.

Keywords: High-intensity lasers, Semiconductor, Candidiasis, Mouth, *Candida albicans*

Introduction

The total number of eukaryotic species on earth is estimated at 7.8 million, and fungi account for approximately 7% of this number (611 000 species).[1] Of all fungi, approximately 600 species are pathogenic.[2] Pathogenic fungi include those that cause relatively mild cutaneous infections (such as dermatophytes and *Malassezia*), severe cutaneous infections (such as *Sporothrix schenckii*), and systemic life-threatening infections (such as *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Candida albicans*).[3] *C. albicans* is a member of the normal microbial flora of the human body. *C. albicans* and other species of *Candida* (in lower amounts) are present in the oral cavity of 75% of the population. While *C. albicans* is the most common species associated with oral infection, accounting for more than 80% of clinical isolates, several other *Candida* species, such as *C. tropicalis, C. glabrata, C. krusei*, and *C. parapsilosis*, may produce clinical infections, some of which can be particularly insensitive or, indeed, resistant to antifungal therapy (Characterization of oral candidiasis and the *Candida* species profile in patients with oral mucosal diseases). Under certain circumstances, they can become pathogenic and cause infections, ranging from superficial skin infections to life-threatening systemic diseases.[4-6] Clinical diagnosis of candidiasis could be difficult because of the lack of specific symptoms and clinical signs. Multilocus enzyme electrophoresis, fragment length polymorphism, electrophoretic karyotyping, random amplified polymorphic DNA, and multilocus sequence typing are known as efficient techniques used for molecular typing of *Candida* species.[4]

Underlying conditions involved in the development of candidiasis include systemic antibiotic therapy with broad-spectrum antibiotics, xerostomia, impaired function of the immune system (secondary to systemic diseases such as diabetes mellitus or use of immunosuppressant), and use of dentures.[7-10] Candidiasis is often confined to the skin or mucous membranes unless the patient has severe immunodeficiency.[11,12] Cultures are negative in

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~50% of invasive candidiasis. Data are emerging for the performance of nonculture tests such as mannan/anti-mannan, *C. albicans* germ tube antibody, 1,3-β-d-glucan, polymerase chain reaction (PCR), and the T2Candida panel in diagnosing both candidemia and deep-seated candidiasis (diagnosing invasive candidiasis). Oral candidiasis, which can often present in patients with mild immunodeficiency, can cause pain and loss of appetite. It is mainly caused by *C. albicans*, although other species such as *C. glabrata, C. tropicalis*, *C. krusei*, and *C. parapsilosis* can also cause candidiasis.[13,14]

Systemic azoles such as ketoconazole, fluconazole, and itraconazole are the main antifungal agents used for the treatment of candidiasis. However, resistance to fluconazole in HIV patients is an emerging problem.[11,15]

Since *C. albicans* mainly causes superficial infections, it often responds to topical treatments. However, the success of topical and local treatments depends on adequate exposure time (2 minutes) of the oral mucosa to the medication. The duration of treatment varies from 7 to 14 days, and treatment should continue for at least 2 to 3 days after the resolution of clinical signs and symptoms. Topical agents in conventional therapeutic doses often have insignificant side effects since they are not absorbed through the gastrointestinal system. Nonetheless, sucrose present in topical agents can be cariogenic, and in case of long-term use, fluoride therapy may be required. Systemic antifungals have advantages such as a single daily dose and simultaneous treatment of all fungal infections in different parts of the human body. Nonetheless, they have more complications.[11]

Nystatin is commonly used for the treatment of oral candidiasi.[11] However, it has side effects such as diarrhea, stomachache, tachycardia, bronchospasm, facial edema, muscle pain, Stevens-Johnson syndrome, itching, burning sensation, and skin rashes.[16-19] Long-term use of nystatin requires optimal patient cooperation and can lead to the development of drug-resistant *C. albicans* species.[7,16,20] Also, it is costly. It is believed that the diluting effect of saliva may adversely affect the efficacy of oral antifungal agents and cause treatment failure or re-infection.[16]

Several different laser types such as diode, erbium, helium neon and CO2 are currently used in dentistry. Optimal effects of laser irradiation on cell metabolism, resolution of inflammation, reduction of edema, enhancement of healing, and pain relief have been reported.[21] The presence of reactive oxygen species (ROS) generating in photodynamic inactivation mechanisms can be damaging to biofilm cells, and the principle of light transmission could be penetrating in the matrix layers of extracellular polymeric substance until reaching the target cells at the base layers of biofilm. Laser therapy at low intensity with a wavelength of 600 to 1070 nm is currently defined as photobiomodulation.[22] Laser therapy can significantly decrease pain, inflammation and edema, induce nerve regeneration, and enhance healing.[23-26]

High-intensity laser therapy (HILT) has recently gained the spotlight as well. It is often used to remove diseased tissue and create an aseptic area.[27,28] HILT is applied for soft tissue surgical procedures such as gingivectomy, ulectomy, ulotomy, frenectomy, and fiberotomy. Better hemostasis, lower rate of postoperative pain and infection, minimal tissue contraction, decreased or no need for suturing, shorter surgical time, less trauma, and less postoperative edema and scarring are among the advantages of HILT.[29] Also, it has been hypothesized that HILT may decrease the number of viruses in vesicle fluid in herpes labialis by causing a local temperature rise.[30]

Considering the gap in information regarding the effect of HILT on *C. albicans*, this study aimed to assess the effect of the high-intensity diode laser on the *C. albicans* colony count.

Materials and Methods

Sample Size Calculation

The sample size was calculated to be 12 in each group from each concentration (a total of 72) according to a previous study,[31] alpha = 0.05, and study power of 80% using STATA 11.

Preparation of Samples

Standard-strain *C. albicans* (ATCC 18 804) was obtained from the Microbiology Research Center of Pasteur Institute of Tehran, Iran. To prepare the fungal suspensions with the desired concentrations, *C. albicans* suspension was diluted by saline (85% NaCl) to obtain a density of 1.5×10^8 cells/mL. The samples were then cultured on Sabouraud dextrose agar containing chloramphenicol. Next, they were incubated at 37°C for 24 hours. Finally, two different concentrations of *C. albicans* colonies containing 10^4 cells/mL and 10^6 cells/mL were prepared. A total of 72 samples (n = 36 from each concentration) were prepared and assigned to three groups (n = 12) of control, nystatin and laser.

The samples were cultured again on Sabouraud dextrose agar by a standard loop and incubated at 37°C for 24 hours. The number of C. albicans colonies on each plate was then counted and reported as colony-forming units/milliliter (CFUs/mL).

Preparation of the Sabouraud Dextrose Agar Culture Medium Supplemented With Chloramphenicol

A total of 65 g of Sabouraud dextrose agar powder (Merck, Germany) was weighed and added to 1 L of distilled water in an Erlenmeyer flask and heated to the boiling point until it became clear. Next, 50 mg of chloramphenicol powder was weighed and dissolved in 10 mL of pure ethanol and added to the melted culture medium. It was then capped with a cotton pellet, wrapped with aluminum foil, and autoclave-sterilized at

121°C and 15 lb/in^2 pressure for 10 minutes. Next, the temperature was allowed to reach 50-55°C and then the culture medium was poured in sterile 10-cm plates with 4-5 mm depth under a hood.

Interventions

In the laser group, the Ga-Al-Ar diode laser (EPIC10; Biolase, USA) with a 940-nm wavelength and 2-W power was irradiated to the surface of samples (both concentrations of *C. albicans*) with a hand-piece with a 0.4-mm tip in non-contact mode from a 1-mm distance at a speed of 1 mm/s (1 mm of samples is irradiated with the laser every second) from six different directions (Table 1). The samples were cultured on Sabouraud dextrose agar and incubated at 37°C for 24 hours. Next, the number of *C. albicans* colonies on each plate was counted by using a loop and reported as CFUs/mL.

In the nystatin (gold-standard) group, 10 cc of nystatin suspension (100 000 IU; Emad, Iran) was added to the samples (both concentrations of *C. albicans*), mixed on a shaker for 30-60 seconds (this time is needed for mixing according to new studies), and the samples were then cultured on Sabouraud dextrose agar and incubated at 37°C for 24 hours. Next, the number of *C. albicans* colonies on each plate was counted by using a loop and reported as CFUs/mL. The total amount of time is 30 s and the total energy is 60 J. The control group did not undergo any intervention.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess the normality of data distribution, which showed normal distribution of data in all groups ($P>0.05$). Thus, the three groups were compared by ANOVA. Pairwise comparisons were performed by Tukey's test. All statistical analyses were carried out by SPSS version 24. A P value <0.05 was defined as statistically significant.

Table 1. Laser Parameters

Parameters	
Type of laser	Diode 940 nm
Emission mode	Continuous
Time on/Time off	30 s
Delivery system	Straight handpiece
Energy distribution	60 J
Peak power	-
Average power	2 W
Spot diameter at the focus	0.4 mm
Focus-to-tissue	-
Spot area at the tissue	-
Peak power density at spot area	10 J/cm^2
Peak power density at the tissue	-
Average power density at the tissue	-
Beam divergence	-
Water irrigation	-
Air and aspirating airflow	-

Results

Table 2 and Figure 1 present the mean colony count in the two concentrations of *C. albicans* in the three groups. The ANOVA test showed a significant difference in the colony count between the three groups in both concentrations (both $P<0.001$).

Table 3 presents the pairwise comparisons by Tukey's test for 10^4 cells/mL concentration that showed that the mean colony count in the nystatin group was significantly lower than that in the control ($P<0.001$) and laser ($P=0.045$) groups. The mean colony count in the laser

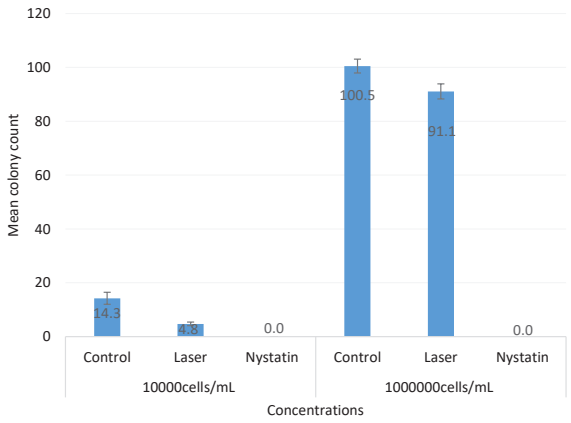

Figure 1. Mean (±Standard deviation) Colony Count in the Two Concentrations of *Candida albicans* in the Three Groups

Table 2. Mean Colony Count in the Two Concentrations of *Candida albicans* in the Three Groups

Concentration	Group	Mean Colony Count	Standard Deviation	*P* Value
10^4 cells/mL	Control	14.25	7.72	<0.001
	Laser	4.75	2.34	
	Nystatin	0.0	0.0	
10^6 cells/mL	Control	100.5	8.89	<0.001
	Laser	91.08	9.69	
	Nystatin	0.0	0.0	

Table 3. Results of Pairwise Comparisons by Tukey's Test

Concentration	(I) group	(J) group	Mean Difference (I-J)	Standard Deviation	*P* Value
10^4 cells/mL	Control	Laser	-9.50	1.90	<0.001
		Nystatin	4.75	1.90	0.045
	Laser	Nystatin	14.25	1.90	<0.001
10^6 cells/mL	Control	Laser	-9.42	3.10	0.013
		Nystatin	91.08	3.10	<0.001
	Laser	Nystatin	100.50	3.10	<0.001

group was significantly lower than that in the control group ($P<0.001$).

Pairwise comparisons by Tukey's test in 10^6 cells/mL concentration revealed that the mean colony count in the nystatin group was significantly lower than that in the control ($P<0.001$) and laser ($P<0.001$) groups. The mean colony count in the laser group was significantly lower than that in the control group ($P=0.013$; Table 3).

Discussion

Considering the gap in information regarding the effect of HILT on *C. albicans*, this study assessed the effect of the high-intensity diode laser on the *C. albicans* colony count. The results indicated that in both concentrations, the mean colony count in the nystatin group was significantly lower than that in the control ($P<0.05$) and laser ($P<0.05$) groups. The mean colony count in the laser group was also significantly lower than that in the control group ($P<0.05$).

A previous study reported results contrary to the result of the recent article like an increase in bacterial proliferation following low-level laser therapy (LLLT).[32] Another study indicated increased proliferation of *C. albicans* as a result of LLLT.[16] Also, Gomes Henriques et al[33] demonstrated that LLLT increased the number of squamous cell carcinoma cells. Nussbaum et al[32] revealed an increase in bacterial proliferation following irradiation of the low-level laser with an 810-nm wavelength and 1 J/cm^2 energy density in continuous mode. Gomes Henriques et al[33] found that irradiation of the 660-nm diode laser with 0.5 and 1 J/cm^2 energy density increased the expression of tumoral markers. These studies are mainly due to the fact that they used LLLT while HILT was applied in the present study. Accordingly, the energy power and density, the laser wavelength, and the duration and mode of laser irradiation were different in the abovementioned studies, compared with the present study. The difference in the laser wavelength is an important parameter that can cause different effects. For instance, visible red light has higher anti-inflammatory effects than infrared light and has greater effects on the growth and proliferation of microorganisms because light with a shorter wavelength has higher absorption by the photoreceptors of bacteria. In the aforementioned studies, despite the greater absorption of laser light, it further stimulated the proliferation of bacteria, probably due to the low energy density of the laser. The reason may be that laser light intensifies the electron transfer chain of the mitochondria and leads to a subsequent increase in ATP production.[34] A previous study showed that HILT in continuous mode decreased the proliferation of fungi, which can be due to the fact that a high-intensity laser exerts photochemical effects by decreasing the stimulation of mitochondrial oxidation and ATP production. Decreased ATP production degrades the fungal cells. However, the required energy for the elimination of all fungal cells was not provided in their study.[35]

The present findings were in agreement with the results of some previous studies. For instance, Maver-Biscanin et al[36] compared the effects of 658-nm (30 mW) and 830-nm (60 mW) lasers on denture stomatitis in 70 patients. They reported comparable antifungal effects of both lasers with antifungal medications. The antifungal efficacy of both lasers was significantly higher than that of the placebo. Similarly, Seyedmousavi et al[37] compared the effects of LLLT with a 685-nm wavelength (50 mW) and 3, 5, 10, and 20 J/cm^2 energy density, and an 830-nm wavelength (400 mW) with 3, 5, 10, 30 and 50 J/cm^2 energy density on fungal culture and found that energy densities > 10 mJ in both 685 and 830 nm lasers significantly decreased the proliferation of *C. albicans* colonies. The results of the past studies were in agreement with the present findings despite the difference in laser wavelengths. The previous studies used lower laser wavelengths, which are better absorbed by the microorganisms, and therefore, lower energy density would be required for their elimination, whereas, in the present study, a 940-nm laser with 1 J/cm^2 energy density was employed which decreased the *C. albicans* colony count but could not eradicate all of them. In the future, it is necessary to test different parameters like energy power and density, laser wavelength, and duration and mode of laser irradiation.

Basso et al[34] evaluated the effect of diode laser irradiation with 5, 10 and 20 J/cm^2 energy density on Streptococcus mutans and *C. albicans* and showed that LLLT decreased the bacterial and fungal colonies, although *Streptococcus mutans* was resistant to laser light when it was accompanied by *C. albicans* colonies. It appears that the interactions between them change the morphology of *S. mutans*, making it resistant to laser light. This finding may explain the resistance of denture stomatitis to laser therapy since it is a polymicrobial condition.[34] Another study reported that the stimulation of polymorphonuclear leukocytes by a laser resulted in the generation of reactive oxygen species, such as hydroxyl radicals and anions, which enhance the fungicidal activity.[38]

In the present study, nystatin eradicated all the fungal colonies; however, HILT decreased the colony count but could not eliminate all of them. This difference may be due to a number of factors such as the power and energy density of the laser, duration of irradiation, or mode of irradiation. By changing these parameters in future studies, we may be able to achieve the complete elimination of fungi with a laser as an alternative to nystatin. Nystatin should be used 3 times a day and has a bitter taste. Thus, its consumption requires patient cooperation and is not pleasant for most patients. Laser therapy is safe given that the laser irradiation protocol and safety measures (such as the use of protective glasses) are strictly followed. It can be performed in one single

session and requires less patient cooperation.

Laser irradiation has anti-inflammatory effects. It decreases the chemotaxis of polymorphonuclears and subsides inflammation as such. Also, it enhances their antimicrobial effects.[38,39] Furthermore, laser irradiation has thermal effects, which result in the degradation of the cell wall of microorganisms, protein denaturation, and eventual death of fungal cells. Laser irradiation also enhances collagen synthesis and wound healing due to the induction of ATP synthesis and the acceleration of the proliferation of fibroblasts.[40]

This study had an in vitro design. Thus, the generalization of the results to the clinical setting must be done with caution. Also, only one laser protocol was evaluated in this study. Future studies are required on different laser powers, energy densities, irradiation times, and modes to find the most effective laser irradiation protocol for the eradication of *C. albicans*. Furthermore, the efficacy of different laser types against *C. albicans* should be evaluated in clinical studies to obtain more reliable results.

Conclusion

The high-intensity laser with a 940-nm wavelength and 2-W power in non-contact mode can significantly decrease the *C. albicans* colony count in vitro, but its effect is lower than that of nystatin.

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Conflict of Interests

No conflict of interest is declared.

Ethical Considerations

This *in vitro*, experimental study was approved by the ethics committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1400.098).

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