Journal of Lasers in Medical Sciences

J Lasers Med Sci 2024;15:e11

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2024.11

In Vitro Effect of Low-level Laser Therapy on *Candida albicans* Colonies Isolated From Patients Undergoing Radiotherapy for Head and Neck Cancer

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Received: September 14, 2023
Accepted: February 21, 2024
ePublished: May 8, 2024

Abstract

Introduction: Studies on head and neck cancer (HNC) patients undergoing radiotherapy have revealed increased numbers of *Candida* spp., leading to oral candidiasis and oral mucositis. The effects of laser therapy on *Candida* spp. have been studied with varied results. This study aimed to investigate the effect of low-level laser therapy (LLLT) on *C. albicans* colonies isolated from HNC patients undergoing radiotherapy.

Methods: This study included a treated group, wherein 11 *C. albicans* isolates were obtained from the saliva of HNC patients undergoing radiotherapy at a dose exceeding 30 Gy. The control groups consisted of an untreated negative control and a positive control treated with nystatin. The treatment groups were subjected to LLLT in continuous mode for 50 seconds at a wavelength of 976 nm, 0.1 W at a dose of 5 J/cm^2 for Laser 1 (L1) group and 0.2 W at a dose of 10 J/cm^2 for Laser 2 (L2) group. The tests were conducted using *C. albicans* inoculum equivalent to a 0.5-McFarland suspension on 96-well plates. All test group inoculums were then cultured on Sabouraud dextrose agar (SDA), and the colony count was assessed at 10^4 CFU/mL.

Results: LLLT at 5 and 10 J/ cm^2 reduced *C. albicans* colonies by 7.01% and 10.94%, respectively, but the reductions were not statistically significant ($P>0.05$). Nystatin eliminated all the colonies.

Conclusion: LLLT shows potential for reducing *C. albicans* colonies in HNC patients undergoing radiotherapy. However, further studies with varied parameters and methods are necessary.

Keywords: Candida; Head and neck cancer; Laser; Radiotherapy.

Introduction

Head and neck cancer (HNC) is the seventh most common cancer in the world, accounting for approximately 10% of all cancers.[1] HNC treatment commonly involves radiotherapy to destroy tumor tissue as much as possible while minimizing damage to normal surrounding tissue.[1-3] More than 60% of HNC patients require radiotherapy, with or without chemotherapy.[4] However, radiotherapy and chemoradiotherapy treatment of HNC present side effects, including acute and chronic toxicity that can manifest as oral mucositis, infectious diseases (oral candidiasis), xerostomia, and/or dysgeusia.[5] Damage to the basement membrane and loss of the mucosal barrier occur at a cumulative dose of 30 Gy.[6] Furthermore, several studies have shown that HNC patients exhibit increased *Candida* spp. after two weeks of radiotherapy, leading to oral candidiasis along with oral mucositis, which causes discomfort to patients and eventual cessation of cancer management.[7]

The most common fungal species found in gargle samples from patients undergoing radiotherapy is *C. albicans*, followed by *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida kefyr*.[8] Sonalika et al[9] reported a significant increase in *Candida* spp. due to radiotherapy. Panghal et al also reported that *C. albicans* is the most significant pathogen in cases of radiotherapy and chemoradiotherapy.[10] Jain et al reported *Candida* spp. increases of 50% in patients undergoing radiotherapy, 75% in patients undergoing chemotherapy, and 81.25% in patients undergoing chemoradiotherapy.[11]

The use of antifungal drugs such as nystatin is a common strategy for the treatment of oral candidiasis. However, its long-term use requires a high level of patient cooperation and regime control to avoid the development of drug resistance in *Candida* spp., especially *C. albicans*.[12] Accordingly, more effective adjunctive techniques are needed to minimize the side effects of radiotherapy.

A possible candidate for this purpose is the use of low-level laser therapy (LLLT).[13] LLLT (or photobiomodulation) is a therapy that utilizes light or

Please cite this article as follows: Hasanah NT, Sufiawati I, Kusumadjati A, Sunardi MA. *In vitro* effect of low-level laser therapy on *Candida albicans* colonies isolated from patients undergoing radiotherapy for head and neck cancer. *J Lasers Med Sci.* 2024;15:e11. doi:10.34172/jlms.2024.11

non-thermal red or near-infrared (NIR) radiation (600-1100 nm) to stimulate cells and tissues through non-ionizing pathways.[14,15] The effects of LLLT administration *in vitro* and *in vivo* on the number of *Candida* spp. colonies have been widely investigated with varied results. The latest *in vitro* study was done by Momeni et al, who showed a significant reduction of *C. albicans* (ATCC 18804) after application of a 940 nm diode laser at 10 J/cm^2 compared to the control group.[12] Meanwhile, in 2008, Khattab performed the treatment of oral candidiasis with an 805 nm diode laser in immunocompromised patients, and then fungal culture was done. The growth of *Candida* spp. was absent after laser treatment compared to antimycotic treatment. Also, the laser can shorten the treatment period.[16] However, there is a lack of studies assessing the effectiveness of LLLT on *Candida* spp. isolates obtained from the saliva of HNC patients undergoing radiotherapy.

This study aimed to determine the *in vitro* effect of LLLT at 5 and 10 J/cm^2 on the numbers of *C. albicans* colonies in HNC patients undergoing radiotherapy exceeding 30 Gy. An untreated group and a nystatin-treatment group served as the negative and positive controls, respectively. Saliva was collected using the concentrated oral rinse method because this method presents all microbes, especially fungal species, in the oral cavity.[17] The number of *C. albicans* colonies on the Sabouraud dextrose agar (SDA) was calculated in units of 10^4 CFU/mL (CFU/plate).

Methods

This research was of a quasi-experimental design using 11 saliva samples from HNC patients undergoing radiotherapy at a dose of more than 30 Gy. The *C. albicans* were isolated and tested *in vitro*. The samples were divided into two treatment groups [Laser 1 (L1): LLLT at 5 J/cm^2; Laser 2 (L2): LLLT at 10 J/cm^2] and two control groups (negative control without intervention; positive control subjected to 100 000 IU/mL nystatin suspension). This research was performed in line with the principles of the Declaration of Helsinki at the Installation of Radiation Oncology of Dr. Hasan Sadikin General Hospital and Laboratory of Microbiology, Faculty of Medicine, Universitas Padjadjaran. Approval was granted by the Research Ethics Committee of Universitas Padjadjaran (No: 540/UN6.KEP/EC/2023). Informed consent was obtained from all human research subjects.

The inclusion criteria were HNC patients older than 18 years who received radiotherapy with a total dose of more than 30 Gy from April to May 2023. The patients whose condition prevented them from adequately rinsing were excluded. We obtained the oral hygiene status of the participants in this research directly before collecting the saliva based on the simplified oral hygiene index (OHI-S) by Greene and Vermillion, which was categorized into three groups: good, fair, and poor.[18] The oral complications of radiotherapy such as oral mucositis, oral candidiasis, xerostomia, dysgeusia, and ageusia were measured subjectively and objectively. Oral mucositis was calculated by using an oral mucositis scoring scale with a Radiation Therapy Oncology Group (RTOG) grade.[19] Xerostomia Inventory (XI) questionnaire and Clinical Oral Dryness Score (CODS) were used to assess the patients' xerostomia.[20]

Saliva samples were taken by gargling with 10 mL of phosphate buffered saline (PBS) solution for one minute, and they were centrifuged at 2000×*g* for 10 minutes. The precipitate was mixed with 1 mL of PBS and homogenized. A 100 µL of the homogenized suspension was cultured by spreading on CHROMagar using an L-spreader. It was incubated for 24-48 hours at 37 °C for presumed identification of *Candida* species. The green colonies indicate *C. albicans* were isolated to perform the subculture on the CHROMagar to confirm the species of *C. albicans* (Figure 1). The *C. albicans* colonies were subcultured on SDA and incubated for 24-48 hours at 37 °C (Figure 2). Colonies were made into a suspension equivalent to 0.5 McFarland (1.5×10^6)

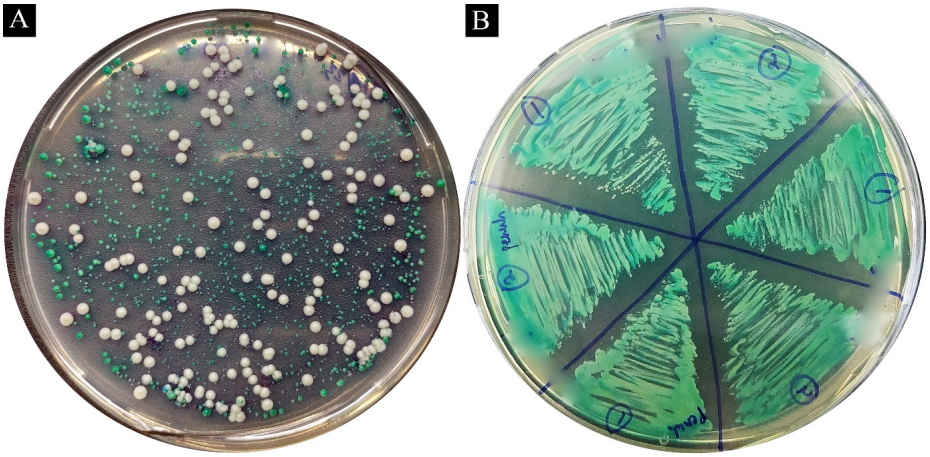

Figure 1. (A) Differentiating Fungal Species Using CHROMagar. (B) Reconfirming *C. albicans* by CHROMagar

(Figures 3A and 3B).

Four suspensions were placed into four wells (each with a volume of 100 µL), which were arranged 4-5 wells apart so that the laser light did not affect the other test groups. The 100 µL suspension was mixed with the 100 µL PBS solution for both L1 and L2 groups. The negative control group samples were 100 µL suspension mixed with 100 µL PBS solution, while the positive control group samples were 100 µL suspension mixed with 100 µL nystatin suspension. The whole suspension was homogenized for 30 seconds.

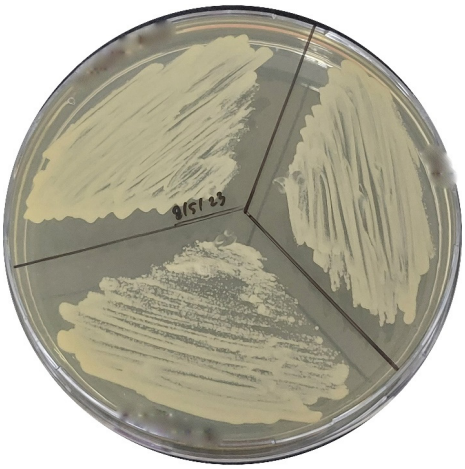

Figure 2. Subculturing on SDA Before Making 0.5 McFarland Equivalent Suspensions

A diode laser (SOLASE-976, Lazon Medical Laser Co., Ltd., China) was used to treat the samples. The treatment group samples were irradiated twice at 5 and 10 J/cm^2 for 25 seconds each time instantly without a time interval with 0.5-1 cm between the beam and the well-plate surface (Figure 3C). The operator used protective glasses during the laser operation (laser parameters are given in Table 1). Positive and negative control groups that had been mixed with a 0.5 McFarland-equivalent suspension were treated for 30 seconds. After the treatment was completed, all suspensions were diluted from 10^{-1} to 10^{-3}. The 100 µL suspensions were subcultured on SDA, and SDA was combined with chloramphenicol for the positive control. Incubation was carried out for 24-48 hours at 37 °C; hereinafter, *C. albicans* colonies were counted by using a colony counter (Figure 4). The MegaStat add-in for Microsoft Excel was applied to analyze the data.

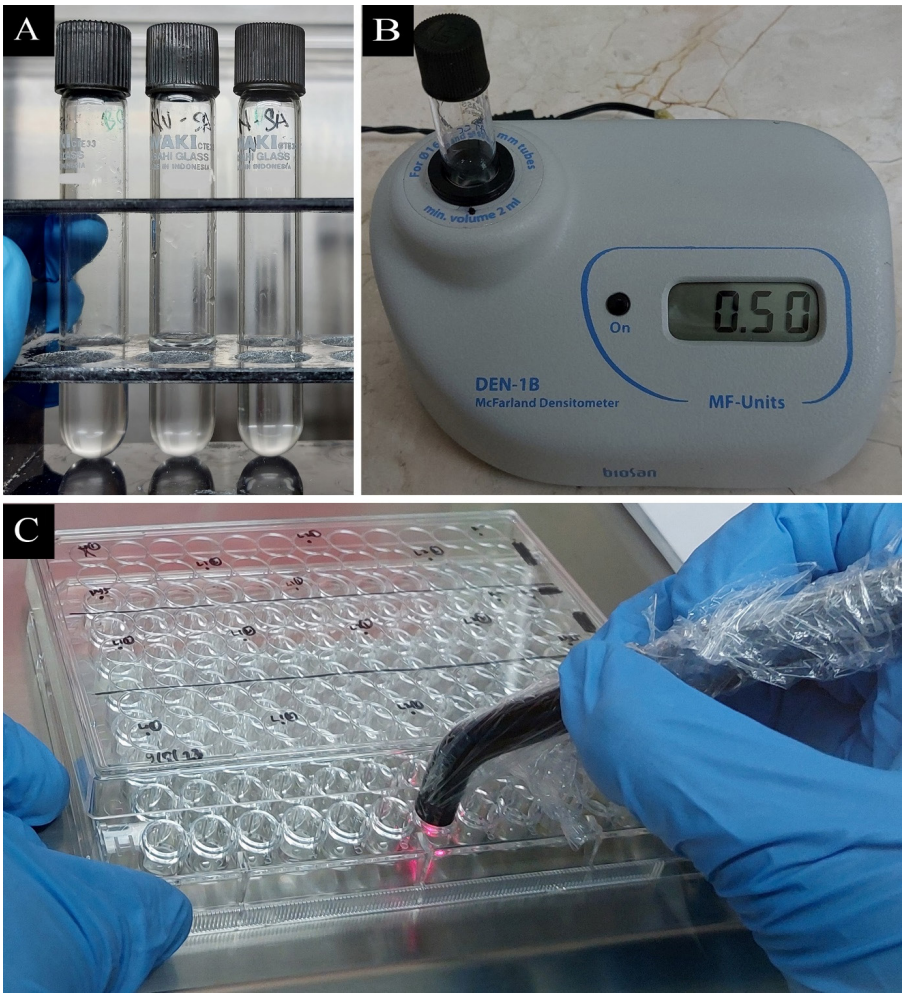

Figure 3. (A) 0.5 McFarland Equivalent Suspensions. (B) 0.5 McFarland Reading on a McFarland Densitometer. (C) LLLT Operation on a 96-Well Plate

Figure 4. Colony Counting Using a Colony Counter

Table 1. Laser Parameters

Parameters		
Type of laser	Diode laser 976 nm	
Emission mode	Continuous wave	
Time on/time off	25 sec for two times: 50 sec in total	
Delivery system	Straight handpiece (biomodulation handpiece)	
Spot diameter at the focus	0.5 cm^2	
Focus spot area	Non-contact of 0.5-1 cm between beam and spot area	
Beam divergence	Collimated	
Water irrigation	-	
Air and aspirating airflow	-	
	Laser 1 (L1)	**Laser 2 (L2)**
Energy distribution	2.5 J	5 J
Peak power	0.1 W	0.2 W
Average power	0.1 W	0.2 W
Peak power density at spot area	5 J/cm^2	10 J/cm^2
Average power density at spot area	5 J/cm^2	10 J/cm^2

Results

This study recruited 11 research subjects (8 men and 3 women) in the age range of 21-70 years. Seven research subjects were suffering from nasopharyngeal cancer, and the other research subjects suffered from tongue, oropharyngeal, laryngeal, or sinonasal cancer. Their total radiotherapy doses at the time of sampling ranged from 32-54 Gy. Oral complications were oral mucositis (63.63%), oral candidiasis (100%), xerostomia (45.45%), dysgeusia (9.09%), and ageusia (72.72%) (Table 2). Moreover, the clinical characteristics of research subjects consist of the parameters of each case presented in Table 3. Table 4 explains the number of *C. albicans* colonies (10^4 CFU/mL) in four test groups. The Spearman test (Table 5) revealed that there was a moderate negative correlation (r = -0.44) between the number of *Candida* spp. colonies and OHI-S ($P = 0.09$). However, the correlation between the number of *Candida* spp. colonies and xerostomia revealed a weak positive correlation (r = 0.29 and $P = 0.19$).

Statistical analysis using ANOVA (Table 6) showed a statistically significant difference between the four groups ($P < 0.05$). These results indicate that the four test groups have different effects on the mean number of *C. albicans* colonies. The L1 group showed a 7.01% reduction in the number of *C. albicans* colonies (average 66.3/71.3), while the L2 group showed a 10.94% reduction (average 63.5/71.3). The nystatin group eliminated 100% of the *C. albicans* colonies (average 0/71.3). The macroscopic view of the resulting samples in the SDA culture medium can be seen in Figure 5.

The T-independent test was conducted to analyze differences in the number of *C. albicans* colonies between each test group (Table 7). The results for the nystatin treatment groups were significantly different from those of the untreated groups, L1 and L2 ($P < 0.05$). The numbers of *C. albicans* colonies in the L1 and L2 groups were not significantly different from those of the control group ($P = 0.44$ and 0.23, respectively). There was no significant difference between the results for the L1 and L2 groups ($P = 0.66$).

Discussion

Candida albicans is the most prevalent fungus that causes oral candidiasis as an oral complication of radiotherapy.[8] The mechanism by which radiotherapy damages the microbiome involves inflammatory processes in the host. First, radiation causes direct tissue oxidation, and the inflammatory process alters the local microenvironment, triggering dysbiosis. Microbiome dysbiosis disrupts the immune system, resulting in the upregulation of pro-inflammatory molecules (Th17) and the downregulation of anti-inflammatory molecules (regulatory T-cells). Second, radiation is toxic, causing cellular and epithelial damage (mucosal fragility).[21,22] This process leads to cell death (apoptosis), followed by ulceration, bacterial translocation, and colonization, thereby enhancing the inflammatory response.[22] Radiotherapy may also alter the composition of the oral microbiota, favoring the onset of oral candidiasis. *Candida* spp. hyphae induce increased production of IL-10 as an immunosuppressive cytokine, which indirectly worsens the local immune response.[4,23]

Another factor that can play a role in the occurrence of oral candidiasis is a decrease in the flow rate due to damage to the salivary glands after radiotherapy.[4,23] Although hyposalivation is a late side effect (complication), the salivary flow rate is reduced by 50%-70% after exposure

to a radiotherapy dose of 10-16 Gy. Radiation induces degradation in amylase activity, bicarbonate level, and pH, as well as a significant increase in saliva viscosity, which may facilitate the transition of *Candida* spp. into a pathogen.[4] Poor oral hygiene can also worsen salivary quality and quantity, increasing opportunistic infections, including oral candidiasis.[23] The results of this study showed that 54.54% of the patients had a poor level of oral hygiene, and as many as 45.45% of the patients experienced xerostomia. However, there was no statistically significant relationship between the number of *Candida* spp. colonies with OHI-S and xerostomia. Our findings were different from the previous studies. Sufiawati et al reported that the fair and poor oral hygiene status in chemotherapy patients significantly developed oral *C. albicans* colonization.[24] In addition, Tarapan et al also found a significant correlation between xerostomia and colonization of *Candida* spp. in post-radiotherapy HNC patients.[23] An impaired salivary flow rate causes several oral complications such as dysgeusia, dysphagia, problems when speaking, oral candidiasis, and caries.[23,25] Our study also found dysgeusia (9.09%) and ageusia (72.72%) as oral complications due to radiotherapy. These complications can interfere with the process of successful therapy, so therapeutic strategies, such as laser treatment, are required for the management of oral complications.

We isolated *C. albicans* from HNC patients undergoing radiotherapy to perform an effect assessment by LLLT. Lasers provide red or NIR light (600 to 1000 nm) at powers from 5 to 150 mW/cm^2. The duration of laser application on the target area typically ranges from 30 to 60 seconds per point. Meanwhile, the therapeutic dose ranges from 0.01 to 10 J/cm^2 based on the Arndt-Schulz law.[26,27] Lasers can be helium-neon (HeNe), neodymium-doped yttrium aluminum garnet (Nd:YAG), gallium aluminum arsenide (GaAlAs), indium gallium aluminum phosphorus (InGaAlP), non-thermal, and non-ablative carbon dioxide (CO_2) diodes.[27] Diode lasers based on semiconductor active gallium and arsenide medium are divided into several wavelength types, including 810-830 nm, 940 nm, 980 nm, and 1064 nm. Laser emission

Table 2. Characteristics of Research Subjects

Characteristics	Number of Subjects	Percent
Gender		
Male	8	72.72
Female	3	27.27
Age		
18-30	2	18.18
31-45	1	9.09
46-59	4	36.36
≥60	4	36.36
Type of cancer		
Nasopharyngeal	7	63.63
Oropharyngeal	1	9.09
Laryngeal	1	9.09
Sinonasal	1	9.09
Tongue	1	9.09
Radiotherapy dose		
>30 Gy	11	100.00
OHI-S		
Good	2	18.18
Fair	3	27.27
Poor	6	54.54
Oral complications due to radiotherapy		
Oral mucositis	7	63.63
Oral candidiasis	11	100.00
Xerostomia	5	45.45
Dysgeusia	1	9.09
Ageusia	8	72.72

Table 3. Clinical Characteristics of Research Subjects

No	Gender	Age (y)	Type of Cancer	Radiotherapy Dose (Gy)	OHI-S	Oral Complication					No. of *Candida* spp. Colonies (CFU/mL)
						Oral Mucositis	Oral Candidiasis	Xerostomia	Dysgeusia	Ageusia	
1	Male	64	Tongue	32	Poor	✓	✓	-	-	-	25
2	Male	38	Nasopharyngeal	34	Poor	-	✓	-	-	✓	>300
3	Female	50	Nasopharyngeal	34	Fair	✓	✓	-	-	✓	>300
4	Male	58	Oropharyngeal	44	Poor	-	✓	-	-	✓	>300
5	Male	64	Nasopharyngeal	52	Fair	✓	✓	✓	-	✓	>300
6	Female	48	Nasopharyngeal	34	Fair	✓	✓	✓	-	✓	>300
7	Female	60	Nasopharyngeal	34	Fair	✓	✓	✓	-	✓	>300
8	Male	70	Laryngeal	52	Good	-	✓	-	-	✓	>300
9	Male	56	Nasopharyngeal	54	Fair	-	✓	-	-	✓	>300
10	Male	21	Nasopharyngeal	40	Good	✓	✓	✓	-	-	>300
11	Male	27	Sinonasal	48	Fair	✓	✓	✓	✓	-	>300

Abbreviation: OHI-S, Oral Hygiene Index-Simplified.

Table 4. Number of *Candida albicans* Colonies in Four Test Groups

No.	Colony Count of *C. albicans* in Units of 10^4 CFU/mL			
	Negative Control	Laser 1	Laser 2	Positive Control
1	85	54	64	0
2	62	61	46	0
3	76	54	49	0
4	56	60	71	0
5	60	52	54	0
6	46	44	57	0
7	106	104	80	0
8	54	65	50	0
9	66	75	67	0
10	96	92	96	0
11	77	68	64	0

Table 5. Analysis Using the Spearman Test on the Correlation Between the Number of *Candida* spp. Colonies With OHI-S and Xerostomia

Variable	Correlation Coefficient (r)	*P* Value
Number of *Candida* spp. colonies and OHI-S	-0.44	0.09
Number of *Candida* spp. colonies and xerostomia	0.29	0.19

Abbreviation: OHI-S, Oral Hygiene Index-Simplified.
* *P* value<0.05: significant.

Table 6. Analysis of Differences in the Number of *Candida albicans* Colonies Using ANOVA

Test Group	Mean	n	Standard Deviation	*P* Value
Negative control	71.27	11	18.63	<0.05*
Laser 1	66.27	11	17.98	
Laser 2	63.45	11	14.93	
Positive control	0.00	11	0.00	

* *P* value<0.05: significant.

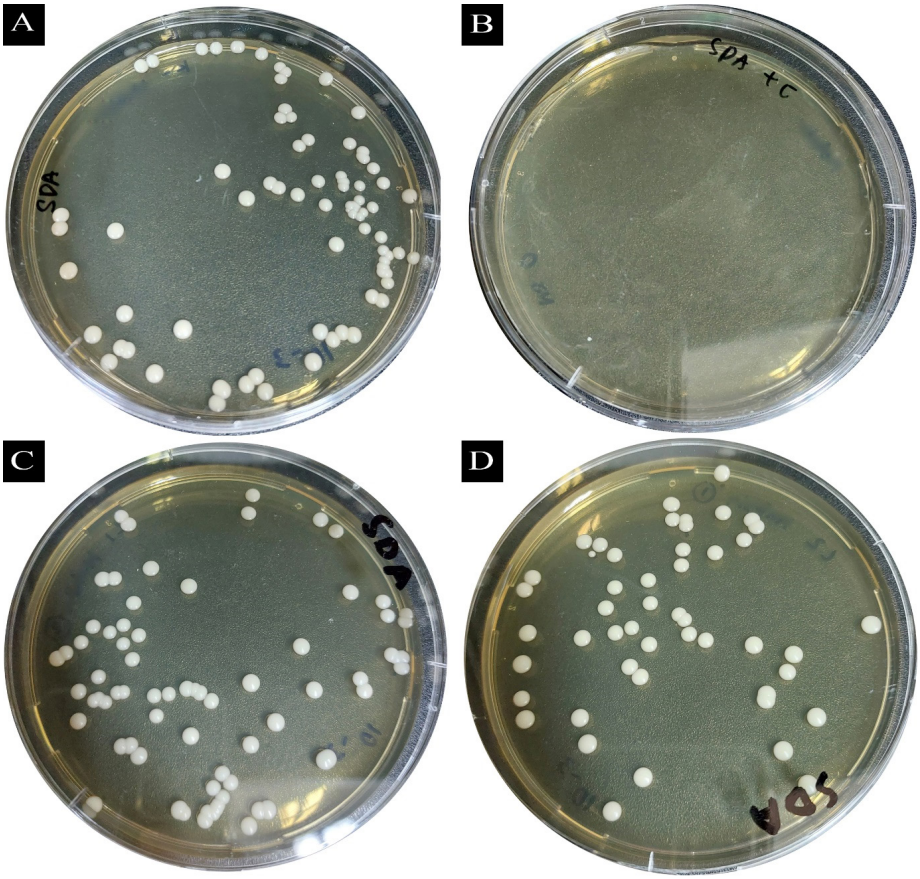

Figure 5. *Candida albicans* Colonies in the 4 Test Groups. (A) Negative control, (B) positive control (C) 5 J/cm² LLLT, (D) 10 J/cm² LLLT

modes are divided into three types, namely continuous-wave mode, gated-pulse mode, and free-running pulsed mode.[26]

This study used a diode laser with a wavelength of 976 nm at doses of 5 and 10 J/cm² for 50 seconds. Our dose selection was based on the Arndt-Schulz law and previous research by Momeni et al and Basso et al. They used doses of 10 J/cm² and 5 J/cm², respectively, which were effective in inhibiting the growth and proliferation of *C. albicans* colonies.[12,28] The continuous emission mode was used because the dissemination of heat energy produced is more stable, preventing excessive heat accumulation in the tissue and thus minimizing tissue damage to host cells.[26,27,29]

The number of *C. albicans* colonies showed significant differences between the four test groups. The positive control (nystatin group) revealed no colony growth; meanwhile, if laser groups were compared to the negative

Table 7. Statistical Analysis Results

Test Group Comparison	*P* Value[a]
Positive control and negative control	
Positive control and laser 1	<0.05*
Positive control and laser 2	
Negative control and laser 1	0.44
Negative control and laser 2	0.23
Laser 1 and laser 2	0.66

* *P* value < 0.05: significant.
[a] *independent* samples *t-test*.

control, the number of *C. albicans* colonies tended to decrease. The results of this study illustrate descriptively that LLLT can reduce the number of *C. albicans* colonies, although the results were not statistically significant for LLLT at a wavelength of 976 nm and doses of 5 and 10 J/cm^2 (Table 6). These results are in line with research by Carneiro et al[30] who examined the effect of LLLT on the number of *C. albicans* colonies with *C. albicans* samples derived from AIDS patients *in vitro*. They observed a decrease in *C. albicans* colonies for a red laser (685 nm) at doses of 6, 8, and 10 J/cm^2 and an infrared laser (830 nm) at doses of 6, 8, 10, and 12 J/cm^2. An increase in the number of *C. albicans* colonies was observed for an LLLT wavelength of 685 nm with a dose of 12 J/cm^2. Statistical results showed no significant difference ($P > 0.05$).[30] Najafi et al also examined the *in vitro* effect of a 940 nm Ga-Al-Ar diode laser on the number of *C. albicans* (ATCC 18804) colonies. They used doses of 38 and 76 J/cm^2 for both 10^4 and 10^6 concentrations, with exposure times of 30 and 60 seconds. The results showed an increase in the number of *C. albicans* colonies at both concentrations ($P > 0.05$).[29]

Other researchers have also examined the effect of LLLT on the number of *C. albicans* colonies using various LLLT parameters. Owlia et al researched the effect of diode laser treatment on *C. albicans* colonies in denture users. Their study used a diode laser with a wavelength of 940 nm, a time of 30 seconds, and a power of 0.1 W. The laser was operated in continuous mode at a distance of 5-10 mm from the surface of the denture, resulting in a significant decrease in *C. albicans* colonies ($P < 0.05$).[31] Seyedmousavi et al investigated the pathogenicity of *C. albicans in vitro* and *in vivo* using laser radiation (685 and 830 nm) at energies of 3, 5, 10, 20, 30, and 50 J. Their results showed that an energy of more than 10 J at both wavelengths significantly affected kinetic growth turbidity ($P \leq 0.05$).[32]

The lasers' mechanism for inhibiting microorganisms is not known for certain, but several mechanisms were described by de Souza da Fonseca et al in 2021.[33] Lasers are known to reduce the number of colonies, increase the number of colonies, or have no effect on microorganisms. Lasers induce photo-acceptor activation that can increase the synthesis of ATP, nucleic acids, and proteins, resulting in cell proliferation and increased colony numbers. The stimulation (excitation) of photosensitizers increases the production of free radicals at cytotoxic levels that can damage molecules. This process inactivates cells and inhibits cell proliferation, which can reduce the number of colonies. If the increase in ATP synthesis is equivalent to the production of free radicals, the laser treatment has no laser effect on microorganisms.[33]

Radiation absorbs chromophores that cause conformational changes in some molecules that produce free radicals, thus triggering disruption or damage to the membranes of bacteria and fungi.[34] On the other hand, some researchers argue that microbial cells are transparent to visible laser light and are not affected by radiation. Sensitizing the cell with a chemical dye before the laser treatment will help absorb the light of the laser wavelength and produce an excited triplet state. It will transfer the energy to the surrounding molecules. This procedure results in the formation of reactive contents such as singlet oxygen, superoxide ions, hydroxyl, and other radicals, which can damage and kill cells directly.[35]

LLLT has therapeutic effects by increasing neo-angiogenesis, collagen fibroblast synthesis, and ATP. LLLT also has fibrinolytic effects, anti-inflammatory effects that block prostaglandin synthesis, analgesic effects through secreting chemotaxis substances that stimulate endorphin release, and fungicidal and bactericidal effects by increasing the amount of interferon and damaging the cell wall that causes protein denaturation in the cytoplasm.[29,34] The specifications of a laser and the biological parameters of the microorganism affect laser treatment effectiveness. Important laser properties of influence include its wavelength, fluence or dose, irradiance, exposure time, emission mode (continuous or pulse), and number or frequency of exposures. The biological factors of the microorganisms involved are those related to metabolic conditions, growth phase, bacterial or fungal species, and culture media conditions.[33]

The results of this study showed that there was an increase in *C. albicans* colonies after being treated with LLLT *in vitro* in three participants with nasopharyngeal cancer and one participant with laryngeal cancer. Increased virulence and colony resistance in these patients may influence this result. However, this assumption cannot be fully proven because further examinations related to mutation patterns cannot be performed. Previous studies showed *C. albicans* altered cell morphology after HNC radiotherapy exposure, sensitivity to antifungal agents, and increased pathogenicity, such as increased phospholipase and proteinase levels.[36-38] Fungal species become resistant and undergo accelerated growth, influencing the virulence profile of the fungi.[37] The prior studies reported that laser therapy can be used as both adjuvant treatment or alternative treatment for treating

oral candidiasis, which is resistant to antifungal therapy, particularly in immunocompromised patients.[39-42] This study has some limitations, including the availability of laser types with different parameters, which are also related to laser exposure time, and the fact that advanced tests were not carried out for mutation patterns. In addition, this study also requires a larger sample size because it uses several clinical parameters.

Conclusion

LLLT at a wavelength of 976 nm and doses of 5 and 10 J/cm^2 reduced the number of colonies of *C. albicans* isolates in the saliva of HNC patients undergoing radiotherapy, but not significantly ($P>0.05$). Future studies should be considered to confirm these findings using different laser parameters, such as extended exposure time, and alternative methods, including the addition of chemical dyes or photosensitizers to LLLT (i.e., photodynamic therapy).

Acknowledgments

The authors thank the members of the Microbiology Laboratory at the Faculty of Medicine, Universitas Padjadjaran, and the Installation of Radiation Oncology at Dr. Hasan Sadikin General Hospital, Bandung, West Java, Indonesia, for their generous assistance. Additionally, the authors extend their thanks to Universitas Padjadjaran for funding this publication.

Authors' Contribution

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Competing Interests

The author reports no conflicts of interest in this work.

Ethical Approval

Informed consent was obtained from all human participants. All protocols in this study were confirmed and approved by the Research Ethics Committee of Universitas Padjadjaran, Bandung, West Java, Indonesia (No: 540/UN6.KEP/EC/2023).

Funding

None.

References

1. Beyzadeoglu M, Ozyigit G, Ebruli C. Basic Radiation Oncology. Berlin: Springer; 2010. p. 1-198.
2. Nishii M, Soutome S, Kawakita A, Yutori H, Iwata E, Akashi M, et al. Factors associated with severe oral mucositis and candidiasis in patients undergoing radiotherapy for oral and oropharyngeal carcinomas: a retrospective multicenter study of 326 patients. Support Care Cancer. 2020;28(3):1069-75. doi: 10.1007/s00520-019-04885-z.
3. Yucel SB, Gural Z, Sahin B, Kadioglu H. Oral mucositis: a crucial problem during radiation therapy. 2014;4(1):226. doi: 10.4172/2167-1222.1000226.
4. Rupe C, Gioco G, Almadori G, Galli J, Micciché F, Olivieri M, et al. Oral *Candida* spp. colonisation is a risk factor for severe oral mucositis in patients undergoing radiotherapy for head & neck cancer: results from a multidisciplinary mono-institutional prospective observational study. Cancers (Basel). 2022;14(19):4746. doi: 10.3390/cancers14194746.
5. Teoh F, Pavelka N. How chemotherapy increases the risk of systemic candidiasis in cancer patients: current paradigm and future directions. Pathogens. 2016;5(1):6. doi: 10.3390/pathogens5010006.
6. Ingrosso G, Saldi S, Marani S, Wong AY, Bertelli M, Aristei C, et al. Breakdown of symbiosis in radiation-induced oral mucositis. J Fungi (Basel). 2021;7(4):290. doi: 10.3390/jof7040290.
7. Belazi M, Velegraki A, Koussidou-Eremondi T, Andreadis D, Hini S, Arsenis G, et al. Oral *Candida* isolates in patients undergoing radiotherapy for head and neck cancer: prevalence, azole susceptibility profiles and response to antifungal treatment. Oral Microbiol Immunol. 2004;19(6):347-51. doi: 10.1111/j.1399-302x.2004.00165.x.
8. Vanhoecke B, De Ryck T, Stringer A, Van de Wiele T, Keefe D. Microbiota and their role in the pathogenesis of oral mucositis. Oral Dis. 2015;21(1):17-30. doi: 10.1111/odi.12224.
9. Sonalika WG, Amsavardani Tayaar S, Bhat KG, Patil BR, Muddapur MV. Oral microbial carriage in oral squamous cell carcinoma patients at the time of diagnosis and during radiotherapy-a comparative study. Oral Oncol. 2012;48(9):881-886. doi:10.1016/j.oraloncology.2012.03.018.
10. Panghal M, Kaushal V, Kadayan S, Yadav JP. Incidence and risk factors for infection in oral cancer patients undergoing different treatments protocols. BMC Oral Health. 2012;12:22. doi:10.1186/1472-6831-12-22.
11. Jain M, Shah R, Chandolia B, Mathur A, Chauhan Y, Chawda J, et al. The oral carriage of *Candida* in oral cancer patients of Indian origin undergoing radiotherapy and/or chemotherapy. J Clin Diagn Res. 2016;10(2):ZC17-20. doi: 10.7860/jcdr/2016/15702.7180.
12. Momeni E, Didehdar M, Sarlak E, Safari M. In vitro effect of a high-intensity laser on *Candida albicans* colony count. J Lasers Med Sci. 2022;13:e59. doi: 10.34172/jlms.2022.59.
13. Sroussi HY, Epstein JB, Bensadoun RJ, Saunders DP, Lalla RV, Migliorati CA, et al. Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. Cancer Med. 2017;6(12):2918-31. doi: 10.1002/cam4.1221.
14. Cerdeira CD, Lima Brigagão MR, Carli ML, de Souza Ferreira C, de Oliveira Isac Moraes G, Hadad H, et al. Low-level laser therapy stimulates the oxidative burst in human neutrophils and increases their fungicidal capacity. J Biophotonics. 2016;9(11-12):1180-8. doi: 10.1002/jbio.201600035.
15. Dompe C, Moncrieff L, Matys J, Grzech-Leśniak K, Kocherova I, Bryja A, et al. Photobiomodulation-underlying mechanism

and clinical applications. J Clin Med. 2020;9(6):1724. doi: 10.3390/jcm9061724.
16. Khattab OS. Treatment of oral candidiasis by 805 nanometer diode laser in immunocompromised patients. Qatar Med J. 2008;17(2):12-6. doi: 10.5339/qmj.2008.2.8.
17. Marsh PD, Lewis MA, Rogers H, Williams D, Wilson M. Marsh & Martin's Oral Microbiology. Marsh & Martin's Oral Microbiology. 6th ed. Elsevier; 2016.
18. Reddy S. Essentials of Clinical Periodontology and Periodontics. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers; 2008. p. 48-9.
19. Liu S, Zhao Q, Zheng Z, Liu Z, Meng L, Dong L, et al. Status of treatment and prophylaxis for radiation-induced oral mucositis in patients with head and neck cancer. Front Oncol. 2021;11:642575. doi: 10.3389/fonc.2021.642575.
20. Villa A, Connell CL, Abati S. Diagnosis and management of xerostomia and hyposalivation. Ther Clin Risk Manag. 2015;11:45-51. doi: 10.2147/tcrm.s76282.
21. Patel M. Oral cavity and *Candida albicans*: colonisation to the development of infection. Pathogens. 2022;11(3):335. doi: 10.3390/pathogens11030335.
22. Zagury-Orly I, Khaouam N, Noujaim J, Desrosiers MY, Maniakas A. The effect of radiation and chemoradiation therapy on the head and neck mucosal microbiome: a review. Front Oncol. 2021;11:784457. doi: 10.3389/fonc.2021.784457.
23. Tarapan S, Matangkasombut O, Trachootham D, Sattabanasuk V, Talungchit S, Paemuang W, et al. Oral *Candida* colonization in xerostomic postradiotherapy head and neck cancer patients. Oral Dis. 2019;25(7):1798-808. doi: 10.1111/odi.13151.
24. Sufiawati I, Pratiwi U, Wijaya I, Rusdiana T, Subarnas A. The relationship between *Candida albicans* colonization and oral hygiene in cancer patients undergoing chemotherapy. Mater Today Proc. 2019;16(Pt 4):2122-7. doi: 10.1016/j.matpr.2019.06.101.
25. Asif M, Moore A, Yarom N, Popovtzer A. The effect of radiotherapy on taste sensation in head and neck cancer patients - a prospective study. Radiat Oncol. 2020;15(1):144. doi: 10.1186/s13014-020-01578-4.
26. Convissar RA. Principles and Practice of Laser Dentistry. Elsevier; 2016. p. 12-25.
27. Robijns J, Nair RG, Lodewijckx J, Arany P, Barasch A, Bjordal JM, et al. Photobiomodulation therapy in management of cancer therapy-induced side effects: WALT position paper 2022. Front Oncol. 2022;12:927685. doi: 10.3389/fonc.2022.927685.
28. Basso FG, Oliveira CF, Fontana A, Kurachi C, Bagnato VS, Spolidório DM, et al. In Vitro effect of low-level laser therapy on typical oral microbial biofilms. Braz Dent J. 2011;22(6):502-10. doi: 10.1590/s0103-64402011000600011.
29. Najafi S, Sheykhbahaei N, Khayamzadeh M, Gholizadeh N. The effect of low-level laser on number of *Candida albicans* colonies in-vitro: a new finding. BMC Oral Health. 2019;19(1):104. doi: 10.1186/s12903-019-0814-5.
30. Carneiro VS, Araújo NC, de Menezes RF, Moreno LM, dos Pereira Santos-Neto A, Gerbi ME. Effect of low-level laser therapy on the fungal proliferation of *Candida albicans*. In: Mechanisms of Photobiomodulation Therapy XI. Vol 9695. San Francisco: SPIE; 2016. p. 109-15. doi: 10.1117/12.2214269.
31. Owlia F, Akhavan Karbassi MH, Motallaei MN. Effect of diode laser on the *Candida albicans* colonization in complete denture wearers. Biointerface Res Appl Chem. 2022;12(3):2745-51. doi: 10.33263/briac123.27452751.
32. Seyedmousavi S, Hashemi SJ, Rezaie S, Fateh M, Esmaeeli Djavid G, Zibafar E, et al. Effects of low-level laser irradiation on the pathogenicity of *Candida albicans*: in vitro and in vivo study. Photomed Laser Surg. 2014;32(6):322-9. doi: 10.1089/pho.2012.3387.
33. de Souza da Fonseca A, da Silva Sergio LP, Mencalha AL, de Paoli F. Low-power lasers on bacteria: stimulation, inhibition, or effectless? Lasers Med Sci. 2021;36(9):1791-805. doi: 10.1007/s10103-021-03258-5.
34. Andraus RA, Maia LP, dos Santos JP, Mesquita AR, Santos TG, Braoios A, et al. Analysis of low-level laser therapy in vitro cultures of bacteria and fungi. Man Ther Posturology Rehabil J. 2015;13:304. doi: 10.17784/mtprehabjournal.2015.13.304.
35. Maver-Biscanin M, Mravak-Stipetic M, Jerolimov V, Biscanin A. Fungicidal effect of diode laser irradiation in patients with denture stomatitis. Lasers Surg Med. 2004;35(4):259-62. doi: 10.1002/lsm.20075.
36. Dambroso D, Svidzinski TI, Svidzinski AE, Dalalio MM, Moliterno RA. Radiotherapy effect on frequency of *Candida* spp. and on virulence of *C. albicans* isolated from the oral cavity of head and neck cancer patients. Rev Ciênc Farm Básica Apl. 2009;30(2):153-9.
37. da Silva EM, Mansano ES, Miazima ES, Rodrigues FA, Hernandes L, Svidzinski TI. Radiation used for head and neck cancer increases virulence in *Candida tropicalis* isolated from a cancer patient. BMC Infect Dis. 2017;17(1):783. doi: 10.1186/s12879-017-2879-6.
38. Khozeimeh F, Golestan Nezhad Z, Dehghan P, Tabesh A, Kheirkhah M, Jamshidi M, et al. Evaluation and comparison of antifungal effect of voriconazole with nystatin on *Candida* species derived from neoplastic patients undergoing maxillofacial radiotherapy. Dent Res J (Isfahan). 2023;20(1):79. doi: 10.4103/1735-3327.382132.
39. Karic VV, Shackleton JL, Howes DG. Laser therapy as an adjunctive therapy for denture stomatitis. S Afr Dent J. 2019;74(9):500-1. doi: 10.17159/2519-0105/2019/v74no9a5.
40. Zhang W, Wu S, Wang X, Wei P, Yan Z. Combination treatment with photodynamic therapy and laser therapy in chronic hyperplastic candidiasis: a case report. Photodiagnosis Photodyn Ther. 2022;38:102819. doi: 10.1016/j.pdpdt.2022.102819.
41. Sobhy N, Talla Eweed H, Omar SS. Fractional CO2 laser - assisted methylene blue photodynamic therapy is a potential alternative therapy for onychomycosis in the era of antifungal resistance. Photodiagnosis Photodyn Ther. 2022;40:103149. doi: 10.1016/j.pdpdt.2022.103149.
42. Scwingel AR, Barcessat AR, Núñez SC, Ribeiro MS. Antimicrobial photodynamic therapy in the treatment of oral candidiasis in HIV-infected patients. Photomed Laser Surg. 2012;30(8):429-32. doi: 10.1089/pho.2012.3225.