

Effect of Diode Laser on the *Candida Albicans* Colonization in Complete Denture Wearers

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Received: 28.05.2021; Revised: 2.07.2021; Accepted: 5.07.2021; Published: 8.08.2021

Abstract: This study aimed to investigate the effect of diode laser on *Candida albicans* colonization in complete denture patients. A total of 40 complete maxillary dentures wearers were studied in this cross-sectional study. Dentures were then randomly divided into two groups. In the first group, as the control group, no intervention was made. In the second group, the mucosal surface of dentures was exposed to laser irradiation by the diode laser with a wavelength of 940 nm with 100 mW for 30 seconds before delivery. Samples were collected from the mucosal surface of the upper dentures on the determined days. All collected colonies were counted. Clinical outcome was evaluated regarding the colonization of *Candida albicans* reported by CFU. The difference in colony counts results between the two groups was evaluated by the Mann-Whitney test. Statistical significance was assumed if $p < 0.05$. The results showed that laser irradiation was significantly effective in reducing *Candida* colonization. The lower density of the colonies was seen in the laser group compared to the control group on the 15th and 60th days after denture delivery ($P = 0.002$) and ($P = 0.003$), respectively. Generalization our data into the clinical setting, it can be proposed that a direct laser-based approach can significantly reduce the colonization of *Candida albicans*. It can also be concluded that laser light at specific wavelengths could be a promising novel strategy for preventing denture stomatitis.

Keywords: *Candida albicans*; stomatitis; denture; complete; laser; diode.

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1. Introduction

An increase in the aging population is related to longer life expectancy [1, 2]. The updated rates of edentulism have been estimated up to 69 percent of the adult population in the world [3]. One of the main demands of the old is removable denture [1]. Although new methods for tooth replacement were developed, complete denture wearers are a large group of geriatrics, yet [4-7]. Regarding the rise of immunocompromised patients, such as those receiving chemotherapy or cytotoxic drugs, fungal infections have increased in recent years [8-12]. One of the most common oral mucosal discomforts in denture wearers is denture stomatitis [1]. *Candida albicans* have the most important role in the development of denture stomatitis. For sure, the presence of this fungus in the mouth is regarded as commensalism. The transition from normal flora to pathogen organisms is related to an imbalance that occurs between the host and the fungus. This percent will be increased to 60-100% in denture wearers [13-17]. Twenty-four hours of prostheses, trauma to the tissues due to inadequate occlusion, poor oral

hygiene, endocrine and immunological disorders, xerostomia, different types of immunodeficiency, allergic reaction to dental materials, trauma, and antibiotic drugs, and malignant diseases are some of the predisposing factors. The main underlying factor for denture stomatitis is denture itself [18].

Candida albicans could stick to both mucosal and denture surfaces as an effective step to the occurrence of denture stomatitis. Treatment of denture stomatitis can be a challenge due to its complicated etiology [19, 20]. Many efforts have been made to use different methods to manage this lesion, from oral hygiene promotion, topical and systemic antifungal agents to denture replacement [18, 21]. Many efforts have been made to use different methods to prevent or treat this lesion. The use of denture liners containing antifungal agents is a helpful method for the treatment of denture stomatitis in a short time. Optical sensitizers combined with photodynamic therapy in some patients were effective [22, 23]. Also, the use of microwaves has been effective for its management [18]. Diode laser, whose effects are based on the decreased porosity of denture tissue surface, has recently been considered to prevent *Candida* colonization [18, 24, 25]. Other studies paid more attention to the treatment of denture stomatitis than prevention [18, 21, 22, 24, 26]. Basso FG *et al.* claimed that LLLT had an inhibitory effect on the microorganisms, and this capacity can be altered according to the interactions between different microbial species [27]. The role of diode laser in the treatment of candidiasis has recently been taken into account. This study aimed to investigate the effect of diode laser on *Candida albicans* colonization in complete denture wearers.

2. Materials and Methods

2.1. Study design.

This study was performed under the precepts of the World Medical Association's Declaration of Helsinki, as adopted in 2013 in Brazil. A total of 40 complete maxillary dentures from patients referred to the Prosthetic Department of Yazd dental faculty were consecutively examined, and those who met inclusion criteria were selected. They were randomly divided into two groups by a random number table. A complete medical and dental history was taken from all of the participants.

2.2. Inclusion and exclusion criteria.

The medical and dental histories of all participants were obtained. No participant in either group had received antibiotics, steroids, or immune therapy or used any antiseptic mouthwash during the study. Subjects who reported a history of any oral and systemic diseases, taking any drugs, or having a previous denture were excluded from this study.

2.3. Sampling.

In the laser group, the tissue surface of dentures was irradiated by the diode laser (A.R.C GmbH Germany) with a wavelength of 940 nm. The laser was operated continuously at a 5–10 mm distance perpendicular to the surface. The laser was used for 30 seconds with air- and water-cooling spray and the average output power was 0.1 W. In the control group, no intervention was made. Samples were obtained by swabbing from the palatal impression surface of upper dentures before delivery & on the 15 & 60 days after repeating irradiation with the same process and cultured on sabouraud glucose agar plates (Figure 1). All collected

yeasts were first counted and then identified by sub-culturing on CHROM agar Candida (CHRO-Magar, France)[28]. Meanwhile, both groups received the same training for denture health. The microscopic morphology was investigated by counting the number of colony counts after being in the incubator for 48 hours.



Figure 1. Candida cultured on sabouraud glucose agar plates.

2.4. Data analysis.

Data were fed into SPSS-17 software. To analyze the normality of data, Kolmogorov-Smirnov and Shapiro-Wilk tests were used. If data were not normally distributed, the Kruskal-Wallis test was run to analyze the mean differences. Then, the Mann-Whitney test was used to analyze the differences between the means of collected colonies. The level of significance for pair comparisons was considered as $P < 0.05$.

2.5. Ethical consideration.

Ethical approval was obtained from the research ethics committee of Shahid Sadoughi University of medical sciences. All the patients signed an informed consent form before the initiation of the research.

3. Results and Discussion

Thirty-three out of the 40 complete denture patients continued the study, 20 patients in the control group and 13 patients in the laser group. The means and standard deviation of the two study groups are presented in Table 1. The laser group composed of 9 men and 4 women, and with a mean and standard deviation of the age was 55.84 ± 11.52 years. The age range was between 41 to 78 years old. The control group included 20 persons (12 men and 8 women). The age range of patients was 32 to 78, and the mean age of 56.35 ± 13.22 years. There was not any significant age difference between the two groups.

Table 1. Comparison of the average number of colony counts in two groups on the 15th & 60th.

Groups at different times	Number	Mean and standard deviation	P-value
Laser 15th	13	370/88+168/08	0/003
Control 15th	20	435/58+357/05	
Laser 60th	13	370/3+61/174	0/002
Control 60th	19	470/12+575/45	

Positive culture of Candida enrolled on the denture of all subjects on the 15 & 60 days after denture delivery; meanwhile, the average colony count in both groups was near zero on the day of delivery. The samples were reported above 1000, converted to 1000 (Table 1). The colony count difference between the two groups was significant (p -value = 0.003) on the 15th. On the 60th day after delivery, one patient did not come back for the culture. Therefore, he was

omitted. The difference in both groups was significant on the 60th day after delivery. (P-value = 0.002)

After primary analysis, the data were assumed as quantitative by chi-square test and cut of point of 500 (Table 2). As shown in the table, there was no significant difference between the two groups on the 15th day. (P-value = 0.438)

Table 2. Frequency distribution of Candida colony counts on the 15th day after laser irradiation.

Colony count	Laser		Control	
	number	number	number	Percent
<500	11	84/6	14	70
≤500	2	15/4	6	30
total	13	100	20	100

P value = 0/438

There was a significant difference between the groups on the sixty days after irradiation. (P-value=0.007)(Table 3).

Table 3. Frequency distribution of candidiasis colony counts on the 60 days after laser irradiation.

Colony count	Laser		Control	
	number	number	number	Percent
<500	11	84/6	7	36/84
≤500	2	15/4	12	63/15
total	13	100	19	100

P-value=0.007

One of the most important concerns of dentists is the incidence and development of denture stomatitis. Numerous methods have been proposed for the prevention and treatment of this lesion. The use of laser irradiation, cryosurgery, electrosurgery, and blade surgery have been successful results [9, 22, 25, 29-32]. In some cases, in addition to improve oral health, the old denture should be replaced. The palatal surface of the denture could act as a shelter for *Candida albicans* than other surfaces. Use of disinfectant on denture surface is recommended in the treatment of denture stomatitis. Microwave radiation has been shown to be an effective disinfectant, but the hardness of the acrylic surfaces may be impacted. Laser irradiation could be used for the treatment of candidiasis and as a preventive method [24, 25, 33-36].

In this study, the mucosal surfaces of dentures were irradiated with a wavelength of 940 nm .it was operated at a continuous mode at a distance of 5–10 mm perpendicular to the surface before delivery. In some literature, both the tissue surface of the denture and palate were irradiated [24, 34, 35]. In other studies, only the palatal surfaces were affected, while in the present study, only the palatal surface of dentures was irradiated [33, 36, 37]. It is worth mentioning that no exposure to participants, doing all steps of study in the laboratory & prevention of multiple visits were some advantages of this method rather than others [24, 34, 35].

In this study, a diode laser with a wavelength of 940 nm with a power of 0.1W for 30b seconds was used, but in the Sivakumar study, the diode laser (wavelength of 980 nm & power of 2 W) irradiated two times for 15 seconds per visit & treatment procedure was completed in two visits. It was used two times in each session [36]. In the Scwinger study, a wavelength of 660nm of diode laser with a power of 30mw and the intensity of 7.5 J / cm² was used in mucosal contact for 10 seconds [37]. In the Biscanin study, two diode lasers with a wavelength of 830 nm with an intensity of 3 J / cm² for 60 minutes and another 685 nm with a power of 30 MW and 3 J / cm² for 10 minutes were used [25] Alves, a 660 nm diode laser with an intensity of

50 J / cm² for 20 minutes used in conjunction with a Photodithazine optical sensitizer [34]. In Fontes, study, a laser with a wavelength of 660 nm 100 MW and energy 4 Jules per cm² [35]. In the Abduljabbar study, a LED with a wavelength of 440 to 460 nm with a power of 24 MW / cm² for a denture and a 260 MW LED with an intensity of 102 MW / cm² was applied the tissue surface [33]. Almost the wavelength & duration of the laser that has been used in the present study were similar to Sivakumar [36]. It led to minimum damage to the denture in removing porosity.

The samples were taken with sterilized swabs and immediately cultured in a dextrose agar. This work has been done before denture delivery and was repeated on the 15th and 60th days later. The relevant expert calculated the colony count after 48 hours remaining in the incubator. Some studies used the diode laser for the treatment of candidiasis. Quite the opposite, there is no report about the use of diode laser to prevent Candida colonization. Sivakumar used exfoliative cytology and PAS dyeing only before denture stomatitis [36]. Scwingel sampled before delivery to the patients, immediately after delivery and on 7th, 15th, and 30 days later [37]. Biscanin was sampled from denture and palate before and after laser irradiation, but Alves study on 15th, 30, and 45 days [25, 34]. Abduljabbar carried out exfoliative cytology, PAS staining, sampling, and culture before delivery and 3 months later [33]. In order to ensure no contamination of the denture, culture before delivery was mandatory. Before delivery, the colony count was not zero in the two cases related to contamination, probably in the laboratory, but the statistical difference was not significant. Of course, the risk of candidiasis after delivery increased over time due to mucosal contact. One person in the control group was missed because she did not come back for the third step of culture. The least time for primary and secondary Candida colonization on the palatal surface of denture is 15th & 60th days with $p=0.003$ and $p=0.002$, respectively, that were selected in the study [34, 37].

In statistical analysis at zero time, there was no significant difference between groups. In other words, they were similar ($p>0.05$). Assuming the quantitative value of the data, the difference between the two groups was significant at 15th days after delivery ($p=0.003$). Analyzing with chi-square & non parametric test, it was concluded that difference was statistically significant only on the 60th day ($p=0.007$). The small sample size was the main limitation of this study that should be considered in future studies.

4. Conclusions

Mycological findings in the present study revealed that applying diode laser with this process could prevent Candida colonization in the long term (60 days) more than short term (15 days). By eliminating microporosity of dentures and improving their quality, Candida colonization and the probability of denture stomatitis could be decreased.

Funding

This study has been supported financially by the Vice-Chancellor for Research and Technology of Yazd Shahid Sadoughi University of Medical Sciences.

Acknowledgment

The authors wish to give thanks to Shahid Sadoughi University of medical sciences for sponsoring this research.

Conflicts of Interest

The authors declare no conflict of interest.

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