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Effect of photodynamic therapy with two photosensitizers on *Candida albicans*

Arash Azizi [a], Zohreh Amirzadeh [a], Maryam Rezai [b], Shirin Lawaf [d,*], Arash Rahimi [c]

[a] Oral Medicine department, Islamic Azad Universty-Dental Branch, Tehran, Iran
[b] Microbiology department, Islamic Azad University-Dental Branch, Tehran, Iran
[c] Biophysics department, Islamic Azad University, Science and research Branch, Tehran, Iran
[d] Prosthodontics department, Islamic Azad University-Dental Branch-Tehran-Iran

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ABSTRACT

Background and Objectives: Oral candidiasis (OC) is an opportunistic infection of the oral cavity most commonly caused by *Candida albicans* (*C. albicans*). Considering the drawbacks of standard treatments with antifungal agents, this study sought to assess the efficacy of photodynamic therapy (PDT) with methylene blue (MB) and indocyanine green (ICG) photosensitizers against *C. albicans*.
Materials and Methods: In this in-vitro, experimental study, 130 samples of *C. albicans* standard suspensions were subjected to various combinations of MB and ICG photosensitizers with and without laser irradiation with different exposure parameters, nystatin and chlorhexidine (CHX) in 13 groups of 10. Samples were cultured in microplates containing Sabouraud dextrose agar medium and colony forming units (CFUs) were counted after 24 h of incubation at 37 °C. Data were analyzed by SPSS version 19.0, one-way ANOVA and Tamhane's test.
Results: The maximum number of CFUs was seen in the control group (mean of 214,200 CFUs with a log value of 5.32) while the minimum values were noted in the laser (808 nm and 100 Hz PRR) plus ICG (mean of 13,460 CFUs and log value of 4.12) and nystatin (mean of 13,940 CFUs and log value of 4.14) groups.
Conclusion: Within the limitations of this in vitro study, the results revealed that laser application (808 nm, 100 Hz PRR) plus ICG caused a significant reduction in *C. albicans* CFUs.

1. Introduction

Oral candidiasis is an opportunistic infection of the oral cavity. *Candida albicans* is the causative agent of the most common form of OC accounting for 60–70% of the cases [1]. Non-pathogenic *C. albicans* is found in the normal microbial flora of the mouth. However, under certain conditions, it may cause OC [2]. *C. albicans* may be activated in subjects with impaired cellular immunity and cause oral infections [3]. Nystatin and amphotericin B are prescribed routinely for treatment of OC. However, due to bitter taste, these medications cause nausea and are not well tolerated by patients [4]. Moreover, *Candida* species are becoming increasingly resistant to some antifungal agents such as fluconazole and fluconazole-resistant *Candida* species have been found in 81% of AIDS patients under treatment for oral candidiasis [5]. Treatment of fungal infections, especially the invasive forms, is always challenging due to limited availability of medications and the risk of development of resistant species. These findings highlight the need for developing novel treatment strategies for fungal infections [6–8].

Photodynamic therapy is a new therapeutic strategy based on the interaction of a non-toxic photosensitizer and a harmless light source. Combination of these two factors in presence of oxygen results in creation of reactive oxygen species (ROS) and triggers a cascade of biological events that leads to apoptosis and death of microorganisms [9]. In other words, in this process, cells are treated with photosensitizers, which make them susceptible to killing following light exposure. Photosensitizers often have minimal inherent cytotoxicity but trigger the formation of ROS following excitation with appropriate-wavelength light. Photodynamic therapy has been successfully used for treatment of neoplasms and is believed to be a promising novel treatment strategy for a number of non-neoplastic conditions [10,11]. However, the efficacy of PDT for treatment of fungal infections has yet to be fully elucidated. Considering the advantages of PDT, this study aimed to assess the efficacy of PDT with MB and ICG photosensitizers and variable laser parameters against *C. albicans*.

2. Materials and Methods

In this in-vitro, experimental study, 130 samples of *C. albicans* standard suspensions were prepared and evaluated in 13 groups of 10. Sample size was selected based on previous studies [12].

* Corresponding author.
E-mail addresses: drarashazizi@yahoo.com (A. Azizi), Amirzadehzohreh@gmail.com (Z. Amirzadeh), maryamrezai_51@yahoo.com (M. Rezai), drshlawaf@yahoo.com (S. Lawaf), arash_dds@yahoo.com (A. Rahimi).

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A 0.5 McFarland standard suspension of standard strain *C. albicans* (ATCC 10231) was prepared and cultured in the solid Sabouraud dextrose agar. After ensuring the purity of culture, a new 0.5 McFarland standard suspension was prepared. The suspension was transferred to a 96-well microplate (0.1 mL in each well) using a sampler. The same amount of photosensitizer (MB or ICG) or sterile saline was also added. All these steps were performed under a laminar hood to ensure sterility and dark environment. Diode laser (A.R.C. laser GmbH, Nurnberg, Germany) at 606 and 808 nm wavelengths was calibrated and irradiated (Fig. 1).The study groups were as follows: (See Figs. 2,3.)

Group one or control group: No photosensitizer, laser or routine medications were used in this group.

Group two: 0.1 mL of ICG photosensitizer was added to the suspension. Laser was not irradiated.

Group three: Sterile saline solution was added instead of photosensitizer and samples were subjected to diode laser irradiation at a wavelength of 808 nm (continuous wave).

Group four: ICG photosensitizer and 808 nm laser (continuous wave) were used.

Group five: Laser at 808 nm wavelength with 100 Hz pulse repetition rate (PRR) without photosensitizer was used in this group. Sterile saline solution was added instead of photosensitizer.

Group six: 0.1 mL of the ICG was added to the suspensions and followed by laser irradiation at 808 nm wavelength with 100 Hz PRR.

Group seven: 660 nm laser (continuous wave) without photosensitizer was used. Sterile saline was added instead of photosensitizer.

Group eight: Laser (continuous wave) at a wavelength of 660 nm was used with MB photosensitizer.

Group nine: Laser (continuous wave) at a wavelength of 660 nm with 100 Hz PRR was used with MB photosensitizer.

Group 10: Laser (continuous wave) at a wavelength of 660 nm with 100 Hz PRR without photosensitizer was used. Sterile saline solution was added instead of photosensitizer.

Group 11: MB photosensitizer without laser was used.

Group 12: 0.1 mL nystatin (100,000 units, Jaber-ebn-Hayan Pharmaceuticals, Tehran, Iran) was added to the samples in this group.

Group 13: 0.1 mL of 0.2% CHX was added to the suspension.

Diode laser exposure setting in groups with ICG included continuous wave mode, 808 nm wavelength, 10 J/cm^2 radiation dose, 100 mW output power, 100% duty cycle and 100 s of radiation time. The same exposure settings were used in the laser groups with 100 Hz PRR along with ICG except that 50% duty cycle was selected with 200 s of radiation time. In groups using MB, exposure settings included continuous wave mode, 660 nm wavelength, 40 mW output power and 100 s of radiation time. In laser groups with 100 Hz PRR and MB photosensitizer, the same parameters were used except that 50% duty cycle was selected and the radiation time was 200 s [13,14].

Next, the suspensions in each well were cultured in microplates containing Sabouraud dextrose agar medium and the results were reported after 24 h of incubation at 37 °C [12]. (Fig. 2,3). In the nystatin and CHX groups, the pour plate technique was used to count the *C. albicans* colonies in the diluted suspension. For this purpose, 0.1 mL of the suspension was poured into a #8 empty sterile plate by a sampler; 25 mL of the cooled sterile Sabouraud dextrose agar medium was added and after closing the lid, the plate was gently whirled to mix the sample and the medium. The plate was then transferred to an incubator. The CFUs were counted after 24 h of incubation at 37 °C [15].(Fig. 4).

Data analysis:

Data were analyzed by SPSS version 19.0 (Microsoft, IL, USA). The mean and standard deviation (SD) of *C. albicans* CFUs as well as the log values were reported for different treatment groups. The difference in the number of *C. albicans* CFUs among groups was analyzed using one-way ANOVA. Pairwise comparison of groups in terms of the number of CFUs was performed using Tamhane's test due to unequal group variances. Type 1 error was considered as 0.05 and $P < 0.05$ was considered statistically significant.

3. Results

The mean and SD of *C. albicans* CFUs and the log values are shown in Tables 1 and 2.

The maximum number of CFUs was seen in the control group (mean of 214,200 CFUs with a log value of 5.32) while the minimum values were noted in the laser (808 nm and 100 Hz PRR) plus ICG (mean of

Fig. 1. Diode laser.