Comparison of Photodynamic Therapy versus conventional antifungal therapy for the treatment of denture stomatitis: a randomized clinical trial

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Abstract

In this randomized clinical trial, the clinical and mycological efficacy of Photodynamic Therapy (PDT) was compared with that of topical antifungal therapy for the treatment of denture stomatitis (DS) and the prevalence of *Candida* species was identified. Patients were randomly assigned to one of two groups (n = 20 each); in the nystatin (NYT) group patients received topical treatment with nystatin (100 000 IU) four times daily for 15 days and in the PDT group the denture and palate of patients were sprayed with 500 mg/L of Photogem®, and after 30 min of incubation, were illuminated by light emitting-diode light at 455 nm (37.5 and 122 J/cm^2, respectively) three times a week for 15 days. Mycological cultures taken from dentures and palates and standard photographs of the palates were taken at baseline (day 0), at the end of the treatment (day 15) and at the follow-up time intervals (days 30, 60 and 90). Colonies were quantified (CFU/mL) and identified by biochemical tests. Data were analysed by Fisher's exact test, analysis of variance and Tukey tests and κ test (α = 0.05). Both treatments significantly reduced the CFU/mL at the end of the treatments and on day 30 of the follow-up period ($p < 0.05$). The NYT and PDT groups showed clinical success rates of 53% and 45%, respectively. *Candida albicans* was the most prevalent species identified. PDT was as effective as topical nystatin in the treatment of DS.

Keywords: *Candida*, light-emitting diode, nystatin, oral candidiasis, photodynamic antimicrobial chemotherapy, porphyrin
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Introduction

Denture stomatitis (DS) is the most common form of oral candidiasis with an overall incidence of 11–65% in complete denture wearers. This recurring disease is characterized by different degrees of inflammation of the mucosa under the maxillary denture, ranging from petechiae to generalized inflammation with papillary hyperplasia [1]. The aetiology of this problem is multifactorial: decreased salivary flow, medication, endocrinopathies, immunosuppression, metabolic and nutritional factors, smoking, increased age of denture, denture trauma, continuous denture wearing, and poor denture hygiene have been implicated [2]. Nonetheless, the denture–palatal interface offers a unique ecological niche for microorganism colonization because of the relatively anaerobic and acidic environment favouring yeast proliferation without any other predisposing factor present.

Candida albicans is the yeast species most frequently isolated in significant quantities from subjects with DS [1–8]. This oral fungal pathogen is able to grow in a variety of mor-

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phological forms, ranging from blastospores to hyphae. The filamentous growth can promote tissue penetration during the early stages of infection [9]. Moreover, on soft and hard surfaces within the oral cavity, *C. albicans* grows as a biofilm, which consists of a complex community of cells embedded in a matrix of extracellular polysaccharide. When cells exist in a biofilm they exhibit phenotypic properties that are distinct from those of planktonic cells and they have increased resistance to antimicrobial agents [10]. Although *C. albicans* is the most prevalent and virulent species of the genus *Candida*, other non-*C. albicans* species are often isolated from acrylic surfaces and the palatal mucosa, such as: *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. pseudotropicalis*, *C. krusei* and *C. guilliermondii*. The emergence of other *Candida* species is important because they may exhibit higher denture surface adherence, and species such as *C. glabrata*, *C. krusei* and *C. lusitaniae* show inherent resistance or intrinsic reduced susceptibility to antifungal agents [11].

Antifungal agents are commonly used to treat DS, but improvement in denture hygiene, discontinuation of nocturnal denture wearing, and eventually relining or replacing the denture are also required. Topical agents such as nystatin and miconazole have been used effectively [12,13]. However, the diluent effect of saliva and the cleansing action of the oral musculature tend to reduce the concentration of these agents to sub-therapeutic levels. Hence, treatment regimens tend to be prolonged and recurrence rates are high. Systemic antifungal agents such as amphotericin B and fluconazole are also effective, but they do not eradicate the microorganisms that colonize the denture [12]. Nonetheless, the major problem associated with the prolonged or recurrent use of antifungal drugs is the development of resistant species [9,11,13].

This makes it necessary to seek new therapeutic approaches. A promising modality is Photodynamic Therapy (PDT), which uses a photosensitizing agent and light of appropriate wavelength. The interaction between the photosensitizer and light in the presence of oxygen produces reactive species, such as singlet oxygen and free radicals, which cause cell damage and death [14,15]. As a consequence of these non-specific oxidizing agents, organisms resistant to conventional antifungal agents could be successfully killed by PDT, and it seems unlikely that they will develop resistance to such a therapy. PDT is effective against oral species and may not promote damage to host cells and tissues [16,17].

Investigations have shown that *Candida* spp. are susceptible to photoinactivation [14–16,18], including resistant strains [19,20]. Previous studies have shown that PDT is effective in reducing *C. albicans* counts in a murine model of oral candidiasis [16] and for denture disinfection [21,22] when a porphyrin was associated with light-emitting diode (LED) light. In a recent case report, five patients with clinical and microbiological diagnoses of DS were successfully treated with PDT [5]. Nonetheless, the clinical effectiveness of PDT in comparison with conventional antifungal therapy in the treatment of DS is not yet known. Hence, the aims of the present randomized clinical trial were to compare the efficacy of PDT with that of topical nystatin in the treatment of DS and to identify the prevalence of *Candida* species.

Materials and Methods

Study design

This was a two group, parallel, randomized clinical trial comparing the effectiveness of PDT and nystatin in the treatment of patients with DS. The procedures carried out in the study were in compliance with the criteria of Resolution 196/96 of the Brazilian Health Ministry, which regulates research involving human subjects. This research was conducted in accordance with the Declaration of Helsinki, and the protocol of the entire project was approved by the Ethics Committee of the Araraquara Dental School (39/2005—1308. 0.199.000-05 SISNEP). All participants were made aware of the objectives of the study and of the probable risks and benefits. All subjects voluntarily entered the study and signed an informed consent form before their enrolment.

Participants and randomization

Edentulous denture-wearing patients attending the Araraquara Dental School for prosthetic treatment were examined for clinical evidence of DS. The exclusion criteria were based on the medical history of each individual, which was checked for factors known to affect carriage of *Candida* spp., such as diabetes, anaemia, immunosuppression and cancer therapy (radiotherapy or chemotherapy). Similarly, individuals who had received in the past 3 months or who were currently receiving treatment with antibiotics, antifungal agents or steroids were excluded. A total of 40 voluntary patients were selected for inclusion in the present study. Medical and dental histories of the patients were recorded, and comprehensive oral examinations were performed by the same investigator. DS was classified according to the criteria proposed by Newton [23]. To create groups of patients that were similar with regard to baseline characteristics that could influence prognosis other than the treatment being considered, namely risk factors, a stratified randomization was used. The following risk factors were considered in this study: age of the dentures, smoking habits,

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medication use, denture hygiene habits and nocturnal wearing of dentures.

Photosensitizer and light sources

The photosensitizer used in this study was a haematoporphyrin derivative (Photogem®, Photogem LLC Co, Moscow, Russia). Solutions of 500 mg/L Photogem were prepared by dissolving the powder in sterile saline immediately before use (pH 6.6) [5,16]. This solution was stored in a sterile spray bottle and kept in the dark. The absorption properties of Photogem were investigated spectrophotometrically in saline solution (Fig. 1). Absorption spectra were obtained using a UV-visible spectrophotometer (Varian Cary 50 Bio UV, McKinley Scientific, Sparta, NJ, USA).

Two LEDs (LXHL-PR09; Luxeon® III Emitter, Lumileds Lighting, San Jose, CA, USA) devices were designed by Instituto de Física de São Carlos (University of São Paulo, São Carlos, SP, Brazil) especially for this investigation. They covered the wavelength range from 440 to 460 nm, with maximum emission at 455 nm (royal blue). One device, which comprised 24 LEDs uniformly distributed throughout the device giving a light intensity of 24 mW/cm^2, was used to illuminate the denture. This device was fitted with three air coolers to prevent the denture from being heated [5,21,22]. The other device, designed to irradiate the patient's palate, comprised ten LEDs uniformly distributed on a circular platform, with a power output of 260 mW [5]. The intensity of light delivered was 102 mW/cm^2, considering a distance of 2 cm from the platform inside the mouth to the deepest area of the palate. Joined to this platform, there was a semiconducting chip known as a Peltier, used to dissipate the heat generated by the LED light. This chip and an air cooler were used to prevent the device from heating. To perform illumination, the platform remained in the patient's mouth with the LEDs facing toward the palate.

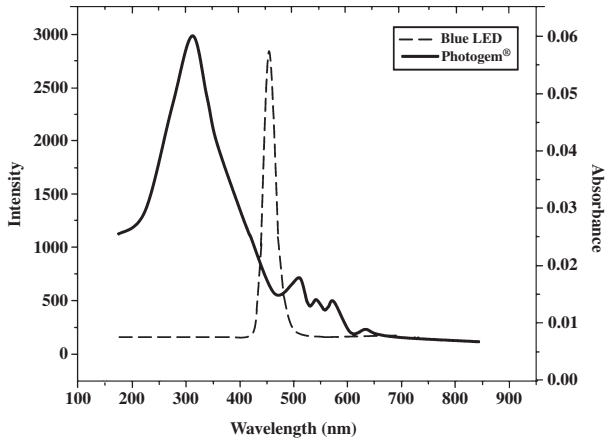

FIG. 1. Absorption bands of Photogem® and intensity of blue (455 nm) LED light.

Interventions

During the 3-month treatment and follow-up of each patient, all subjects were instructed to brush their dentures with coconut soap [24] followed by toothpaste, after every meal and before going to sleep. They also received instructions to immerse the dentures in filtered water overnight. According to the stratified randomization list, the patients were randomly assigned to one of two treatment groups of 20 subjects each. In the nystatin (NYT) group, patients were submitted to topical antifungal treatment with nystatin oral suspension 100 000 IU. Each patient received the medication and was instructed to swish it for 1 min, gargle, and then expectorate it four times daily for 15 days. In the PDT group, each patient had his/her maxillary denture and palate individually submitted to PDT as described previously [5]. Approximately 5 mL of photosensitizer was sprayed on the inner and outer surfaces of each denture. After this, the denture was placed in a transparent plastic bag and left in the dark for 30 min (the pre-irradiation time). To perform illumination, the denture was placed inside the LED device and irradiated for 26 min (37.5 J/cm^2). The denture was centred within the device and surrounded by LEDs during illumination. In this way, both the inner and outer denture surfaces were illuminated. The palate was also sprayed with photosensitizer and illuminated after 30 min of pre-irradiation time. For palate irradiation, the other LED device was handled by the investigator: the platform with LEDs was placed in the patient's mouth and the palate was illuminated for 20 min (122 J/cm^2). PDT was performed three times a week for 15 days (six sessions) in each patient.

Outcomes

The primary outcomes of interest were the *Candida* colony counts from the palates and dentures surfaces, quantified as CFU/mL, and severity of infection of the palatal mucosa, classified according to the criteria proposed by Newton [23] (0, absence of palatal inflammation; or Types I, II and III), measured before treatments (baseline), at the end of treatments (day 15), and at follow-up (days 30, 60, and 90). The second outcome was the prevalence of *Candida* spp. identified in the two groups of treatments at the same time intervals.

Clinical and mycological procedures

For each patient of both groups, oral swab samples were collected from the palatal mucosa and the tissue surface of the maxillary denture as previously described [5]. Each swab

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was placed in a test tube containing 5 mL of 0.9% sterile saline and vortexed for 1 min to suspend the organisms from the swab. Aliquots of 50 μL from this suspension were spread plated on CHROMAgar Candida (Probac do Brasil Produtos Bacteriológicos Ltda., São Paulo, SP, Brazil) and incubated at 30°C for 5 days. Serial ten-fold dilutions from 10^0 to 10^{-3} were plated onto Sabouraud Dextrose Agar (SDA; Acumedia Manufacturers Inc., Baltimore, MD, USA) with 5 mg/mL chloramphenicol. The SDA plates were incubated at 37°C for 48 h. Colonies on SDA were quantified using a digital colony counter (CP 600 Plus; Phoenix Ind. Com. Equipamentos Científicos Ltda., Araraquara, SP, Brazil) and the CFU/mL were determined. To document the clinical response to treatment, standard photographs of the palate of all patients were taken at these same time intervals (days 0, 15, 30, 60 and 90). All the photographs were taken with the same digital camera (Sony Cyber-Shot DSC-F717; Sony Corporation, Tokyo, Japan), by the same operator and under the same conditions (place, light, angle and patient position) to facilitate their reproducibility. Colonies on CHROMAgar Candida were presumptively identified by colony colour and submitted to biochemical tests to confirm all identifications. For this purpose, one colony of each colour type on CHROMAgar Candida was transferred to fresh SDA for determination of purity. After 48 h at 37°C, yeast isolates were identified using the following biochemical tests: carbohydrate assimilation pattern using the ID32C system (bioMérieux, Marcy-l'Etoile, France) and morphological characteristics produced on corn meal agar with Tween-80. In addition, green colonies on CHROMAgar Candida were submitted to a hypertonic Sabouraud broth test for discriminating *C. albicans* and *C. dubliniensis* [5]. All microbiological procedures were carried out by the same operator.

Statistical analysis

The CFU/mL values were logarithm transformed to achieve a normal distribution. However, unlike the data obtained from palate, the distribution of the transformed data obtained from dentures did not follow a normal distribution. Because the patients were evaluated over time, a non-parametric test was not considered appropriate to analyse these paired data so a random-effects statistical model for longitudinal data was used. With this model, data from dentures were transformed $(\ln(\text{CFU/mL}))^{3/2}$ to ensure normality of residuals. The values obtained from palates and dentures were analysed separately. The factors 'treatment group' (NYT or PDT), and 'time' (days 0, 15, 30, 60 and 90) were submitted to analysis of variance and $p < 0.05$ taken as significant. When differences were found, Tukey's *post hoc* test was implemented. Clinical significance between groups was determined by the effect size, which was determined by taking the standardized mean difference in ln(CFU/mL) between pre-treatment and post-treatment results for each group and dividing by the standard deviation of the pre-treatment result. To interpret the resulting number, this general guide was used: <0.1 = trivial effect; 0.1–0.3 = small effect; 0.3–0.5 = moderate effect; and >0.5 = large difference effect [25].

Fisher's exact test was used to assess differences in the degree of inflammation of the palate in relation to the predisposing factors, and significance was assumed when $p < 0.05$.

The standard photographs were evaluated by two independent observers blinded to the treatment groups, period of examination, risk factors and patients' identity. These observers were instructed to classify each photograph of the palate as healthy or with DS type I, II or III. The κ test was used to assess the agreement between the observers and $p < 0.05$ was considered significant.

Results

Patients and predisposing factors

Table I shows that in both groups the number of female patients was higher than that of male patients, the mean age of dentures was >10 years, most patients took medication regularly, and few smokers participated. Before the study began, a large percentage of patients wore their dentures at night and at least 50% of the patients from the two groups showed poor denture hygiene habits.

TABLE I. Distribution of the predisposing factors (age and gender of the subjects, age of maxillary denture, medication use and smoking) for both treatment groups

Group	Age of subjects (years)	Gender of subjects		Age of dentures (years)	Drugs			Smoking
		Female	Male		AH	Others	None	
NYT	41–78 (62.45)	15	5	2–47 (18.55)	9	15	5	2
PDT	43–80 (61.25)	13	7	1–44 (14)	9	14	6	4

AH, antihypertensive (most used drug); NYT, nystatin therapy; PDT, photodynamic therapy.
Mean values are given in parenthesis.

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On day 0, the distribution of patients with DS types I, II and III in the NYT group was 20, 65 and 15%, respectively, and for the PDT group it was 25, 50 and 25%, respectively. The Fisher's exact test showed that only the age of the dentures was significantly associated with the severity of inflammation of the palate (p 0.045). There was no significant association between the other predisposing factors (age, gender, drugs and smoking) and the degree of inflammation.

Mycological efficacy

Statistical analysis showed no significant difference between the CFU/mL values obtained from NYT and PDT groups during all the time intervals of this study. After this, the CFU/mL values from both groups were evaluated together at each time of evaluation (days 0, 15, 30, 60 and 90). For dentures and palates, a significant reduction in ln(CFU/mL) mean values was observed on days 15 and 30 compared with baseline (day 0) (Table 2). This reduction was higher on day 15 than on day 30. At follow-up (days 30, 60 and 90), a significant increase in $(\ln(\text{CFU/mL}))^{3/2}$ mean values obtained from dentures was verified compared with the end of treatment (day 15). For palates, a significant increase in ln(CFU/mL) mean values was found only on day 60 of the follow-up period when compared with the end of treatment (day 15). In Table 2 it can also be observed that the effect size of treatments was large for the palates of patients in the NYT group (1.11) and moderate for the palates of patients in the PDT group (0.39). For the dentures, the effect size was very large for both groups (NYT group = 2.62; PDT group = 1.45).

At baseline (day 0), 97.5% of the swabs taken from the entire fitting surface of the dentures and 70% of the swabs taken from the palatal mucosa produced yeast growth on agar plates. Throughout this investigation, the CFU/mL values from the palates of patients from both groups were lower than those from dentures and a large number of null values were obtained from the palates. This could be attributed to the swab sampling method used for recovering *Candida* cells.

Clinical efficacy

Evaluation of the photographs showed high levels of agreement (p <0.001) between the observers for every time of examination: on day 0, $\kappa = 0.907$ (standard error (SE) = 0.164); on day 15, $\kappa = 0.859$ (SE = 0.120); on day 30, $\kappa = 0.834$ (SE = 0.118); on day 60, $\kappa = 0.852$ (SE = 0.126); and on day 90, $\kappa = 0.875$ (SE = 0.126). These κ coefficients are considered 'almost perfect', according to the recommendations of Landis and Koch [26]. Once a high level of inter-rater reliability was found, the percentage of patients showing cure or improvement of the palatal inflammation was determined. Clinical success (cure or improvement of the palatal inflammation) was considered for each patient whose degree of inflammation at the end of the treatment (day 15) was absent or lower than it was at the baseline (day 0). Hence, for the NYT group, 53% of the patients obtained clinical success, while 41% and 6% showed no alteration and worsening (failure of the treatment), respectively, of the palatal inflammation. For the PDT group, 45% of the patients achieved clinical success (Fig. 2a–c) and 55% showed no

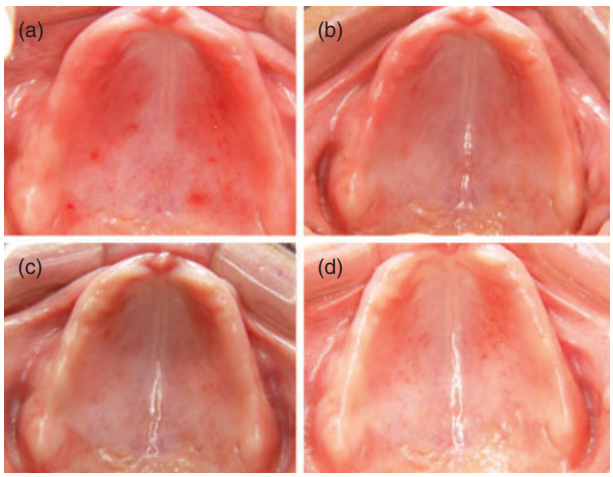

FIG. 2. Palate of patient from photodynamic therapy (PDT) group. (a) Denture stomatitis (DS) type II (day 0); (b) no inflammation (day 15); (c) no inflammation (follow-up time interval, day 30); (d) DS type I (follow-up time interval, day 60).

TABLE 2. Mean values and standard deviation (in parenthesis) of *Candida* colony counts in ln(CFU/mL) from the palates and dentures following treatments

Group	Location	Period					Effect size
		Day 0	Day 15	Day 30	Day 60	Day 90	
NYT	Palate	4.51 (3.52)	0.61 (1.51)*	1.78 (3.32)*	2.77 (3.50)†	2.15 (2.84)	1.11
	Denture	30.88 (7.90)	10.19 (12.85)*	19.33 (15.79)* †	21.41 (18.43)†	19.84 (19.13) †	2.62
PDT	Palate	3.73 (2.95)	2.57 (3.33)*	3.02 (3.59)*	2.71 (3.45) †	2.14 (3.48)	0.39
	Denture	32.23 (12.86)	13.60 (14.64)*	28.09 (14.09)* †	27.06 (14.25)†	28.70 (14.70)†	1.45

NYT, nystatin therapy; PDT, photodynamic therapy.
*Significantly different from baseline (p <0.05); †significantly different from day 15 (p <0.05).

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alteration in the palatal inflammation. During the follow-up (days 30, 60 and 90), recurrence of the palatal inflammation was observed in 75% and 78% of the patients in the NYT and PDT groups, respectively, who had previously obtained clinical success (Fig. 2d).

Prevalence of *Candida* spp.

Candida albicans was the commonest species found on the dentures and palates of patients during all the periods of this investigation. *Candida tropicalis* was the next most common yeast, followed by *C. glabrata*. Other species were observed less frequently (Table 3). At the end of the treatment (day 15) it was verified that the number of isolates of *C. albicans*, *C. tropicalis* and *C. glabrata* was reduced by 50%, 90% and 62.5%, respectively in the NYT group and 50%, 45.5% and 71.4%, respectively, in the PDT group compared with the baseline (day 0) (Table 3). However, at the follow-up (days 30, 60 and 90) the number of these isolates increased compared with the end of the treatments (day 15). On several occasions, more than one species of yeast was isolated from the dentures and palates (23.9% and 21.7% of swabs from NYT and PDT groups, respectively). The combination of *C. albicans* and *C. tropicalis* was the most prevalent of all yeast mixtures in the NYT group (8.7%) and the second most prevalent in the PDT group (7.6%). Whereas, the association of *C. albicans* and *C. glabrata* was the most prevalent in the PDT group (8.6%) and the second most prevalent in the NYT group (7.6%). The yeast mixture of *C. albicans*, *C. tropicalis* and *C. glabrata* was detected in both groups (4.3% and 1.5% in the NYT and PDT groups, respectively). Other yeast combinations were also found, but at lower rates. Mixed-species populations were observed in 60% of the patients from the NYT group and in 70% of the patients from the PDT group on any day of evaluation, but more often at the baseline and follow-up than at the end of the treatment (Table 4). When compared with baseline (day 0), reductions of 50% and 54% in the mixed species were verified at the end of treatments (day 15) for NYT and PDT groups, respectively.

TABLE 4. Number of isolates of mixed-species populations found on dentures and palates of patients in the NYT and PDT groups at the baseline (day 0), at the end of the treatments (day 15) and at the follow-up (days 30, 60 and 90)

	Days				
Group	0	15	30	60	90
NYT	14 (28.6)	7 (14.3)	10 (20.4)	10 (20.4)	8 (16.3)
PDT	13 (30.2)	6 (14)	5 (11.6)	12 (27.9)	7 (16.3)

NYT, nystatin therapy; PDT, photodynamic therapy.
Percentage values are given in parenthesis.

Discussion

The present investigation compared the clinical and microbiological efficacy of PDT with that of conventional antifungal therapy in patients with DS. The microbiological results showed no significant difference between PDT and conventional antifungal therapy in the inactivation of *Candida* spp. At the end of both treatments (day 15), a significant reduction in CFU/mL recovered from dentures and palate was found in comparison with baseline (day 0). This result is in agreement with a previous case report in which reduction of CFU/mL of five patients with DS was observed after six sessions of PDT [5]. Interestingly, on day 30 of the follow-up period (15 days after the end of both treatments), the mean values of CFU/mL were still significantly lower than those at baseline. This could be explained by the patients' compliance with denture and oral hygiene advice given during this research. Although not sufficient to treat DS [24,25], denture and oral

TABLE 3. Number of isolates of each species found on dentures and palates of patients in the NYT and PDT groups on each day of evaluation

	Day 0		Day 15		Day 30		Day 60		Day 90		Total	
Species	NYT	PDT	NYT	PDT	NYT	PDT	NYT	PDT	NYT	PDT	NYT	PDT
Candida albicans	36	26	18	13	21	22	25	18	19	20	119 (63.3)	99 (54)
C. tropicalis	10	11	1	6	5	5	6	8	6	8	28 (15)	38 (21)
C. glabrata	8	7	3	2	6	4	6	5	4	4	27 (14.4)	22 (12)
C. lusitaniae	2	0	0	0	0	0	0	2	0	0	2 (1)	2 (1)
C. parapsilosis	3	0	0	0	1	0	0	0	0	0	4 (2)	0
C. rugosa	0	1	0	1	0	0	0	0	0	1	0	3 (1.6)
C. guillermondii	0	0	2	0	0	0	0	1	0	0	2 (1)	1 (0.5)
Cryptococcus humicola	0	4	3	3	1	3	1	4	0	3	5 (2.7)	17 (9)
Rhodotorula glutinis	0	0	0	0	0	0	1	0	0	0	1 (0.5)	0
Cryptococcus albidus	0	1	0	0	0	0	0	0	0	0	0	1 (0.5)
Kloeckera apis/apiculata	0	0	0	1	0	0	0	0	0	0	0	1 (0.5)

NYT, nystatin therapy; PDT, photodynamic therapy.
Percentage values are given in parenthesis.

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hygiene are essential to maintain a low level of microorganisms on dentures, as well as oral health.

Although several *in vitro* investigations have confirmed the potential of PDT to inactivate *Candida* spp. [14–16,18–22], this is the first clinical trial to evaluate the effect of PDT on the treatment of *Candida* infection. Although Teichert *et al.* [20] stated that mice with oral candidiasis were treated using PDT, they only observed a dose-dependent eradication of *C. albicans* from the oral cavity using methylene blue and laser irradiation. However, the regression of lesions, which is an important clinical sign of treatment, was not evaluated by these authors. Furthermore, the association of Photogem with LED light showed a significant reduction in the CFU/mL of *C. albicans* recovered from the tongues of mice with oral candidiasis and no adverse effects were observed on the tissues of the tongue [16]. Nevertheless, complete eradication of yeast was not achieved. A previous *in vivo* study also showed that PDT, using approximately the same parameters as this investigation, was not toxic to the rat palatal mucosa when 500 mg/L of Photogem was associated with 144 J/cm^2 of blue LED light [17]. Hence, potential toxic or adverse effects of PDT on palatal tissue may seem unlikely. In fact, it is difficult to compare the results of this clinical trial with those of *in vitro* studies and animal models because the oral environment of humans is different, and factors such as microbiota and biofilm composition, salivary flux, oral hygiene and food habits might influence the response to PDT. Nonetheless, the outcome of the present investigation suggests that PDT may be an alternative method for inactivation of *Candida* spp. in patients with DS.

During the follow-up period (days 30, 60 and 90), the mean values of CFU/mL recovered from dentures were significantly higher than those found at the end of the treatment (day 15). This result was expected because progressive recolonization of dentures starts after the end of the treatment. On the other hand, only on day 60 of the follow-up period was the mean value of CFU/mL recovered from the palate significantly higher than that at the end of treatment (day 15). The results obtained during the follow-up period may be explained by the high affinity of *Candida* spp. to adhere to and colonize acrylic surfaces. Virulence factors of *Candida* spp., such as cell-surface hydrophobicity [27] and ability to form biofilm [10], increase its adherence to acrylic surfaces. Other investigations have also demonstrated that *Candida* spp. are primarily found on the tissue surface of the denture rather than on the palatal mucosa of patients with or without DS [5,25,28]. This result corroborates that of the present study, because the palatal cultures evidenced lower CFU/mL mean values than the denture cultures for all the time intervals evaluated. The low level of CFU/mL recovered can be partially attributed to the sample technique (swab sampling) employed in the present investigation. An *in vitro* study showed that *C. albicans* was able to invade a reconstituted human oral epithelium over a period of 48 h through hyphal penetration into the superficial epithelium together with features of cellular internalization of yeasts [29]. Therefore, a delicate cotton swab used in this study for recovering yeast may underestimate the real burden of *Candida* spp. present on the palatal mucosa and denture surface. Hence, another sampling technique, such as an oral rinse method using saline or sterile water, would have been valuable to monitor the overall *Candida* burden in the oral cavity of the patients at baseline and at subsequent evaluations.

In the present study, a higher percentage of clinical success was verified in the NYT group (53%) than in the PDT group (45%). Nystatin would have also reduced *Candida* cells on the tongue and buccal mucosa in patients from the NYT group, which could justify the higher percentage of success in this group. On the other hand, no antimicrobial treatment of the dentures was performed in this group, which could have contributed to the rate of clinical failure obtained (47%). The high rate of clinical failure observed in the PDT group (55%) might be attributed to the recolonization of the palatal mucosa after PDT by *Candida* cells from other sites of the mouth, such as tongue, particularly mid-dorsum, and buccal mucosa. In addition, the clinical conditions in which PDT was performed, such as treatment time (three times a week for 15 days, a total of six sessions) and the parameters used (only one type and concentration of photosensitizer and light fluence) might also explain the rate of clinical failure observed in this group. A longer treatment period might achieve a better resolution of palatal inflammation. Further trials are necessary to evaluate the effect of additional parameters of PDT, such as other types and concentrations of photosensitizer and light fluences, in an endeavour to find a higher clinical success rate.

In the present investigation, the clinical failure rates are not in agreement with the microbiological results obtained, i.e. a reduction in CFU/mL values was observed even in patients who showed no improvement in palatal inflammation. This corroborates the findings of Barbeau *et al.* [1], and Zomorodian *et al.* [8] who found no significant relationship between DS and number of yeast colonies. Furthermore, other reports have demonstrated that healthy denture wearers were also *Candida* carriers [3,6,22,28]. Although both treatments resulted in a significant reduction in values of CFU/mL from dentures and palates in this study, and given that the aetiology of DS is multifactorial, a more effective treatment should be directed towards all aetiological factors involved in this pathology.

This investigation demonstrated that among the patients who showed clinical success, a high rate of recurrence was

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found in the follow-up period (75% for NYT group and 78% for PDT group). Recurrence of infection after the conclusion of treatment has often been reported [4,5,12], because the tissue surface of the acrylic resin denture acts as a reservoir that harbours microorganisms, and is therefore, a potential source of re-infection of patients. Replacement of dentures is also necessary for complete resolution of DS [6], especially when dentures are very old. The present study also showed that the age of dentures was the only predisposing factor significantly associated with the degree of inflammation of the palate. The age of maxillary denture of 52.5% of patients was over 12 years, which could have contributed to the proliferation of yeasts on denture surfaces at follow-up. This result is in agreement with that of Neppelenbroek *et al.* [25], Zomorodian *et al.* [8] and Figueiral *et al.* [2], who found that the time of denture use was related to DS. From this standpoint, the provision of new dentures should also be considered during the management of DS.

The outcomes of the present study showed that *C. albicans* was the predominant species isolated from the dentures and palates of patients from the NYT and PDT groups (63.3% and 54%, respectively). This finding is in agreement with previous reports [2,3,6–8,22,28]. The next most common species found was *C. tropicalis* followed by *C. glabrata* in both groups. Investigations have also observed that *C. tropicalis* was the second most prevalent species isolated from the oral cavity [6,7]. However, other studies have found that *C. glabrata* was the most common yeast after *C. albicans* [2–4,8,22,28]. The presence of non-*C. albicans* species is a cause for concern because they are able to cause infection and are frequently resistant to antifungal agents. In the present study, considerable rates of reduction in the number of isolates of *C. albicans*, *C. tropicalis* and *C. glabrata*, as well as other yeasts species, were verified at the end of the treatments. This result demonstrated that these species were susceptible to nystatin and PDT, except *C. tropicalis*, which showed higher susceptibility to nystatin (90% of reduction) than PDT (45.5% of reduction). Nonetheless, the increased number of these isolates observed in the follow-up period when compared with the end of the treatments may be attributed to recolonization of the mucosa and denture.

During all the time intervals of this investigation, 60% and 70% of the patients from NYT and PDT groups showed mixed-species populations. In other studies, more than one yeast species was found in 27.27% [3], 14.4% [7] and 48.5% [28] of subjects without DS, and 38.5% [3] and 32.5% [7] of individuals with DS. The complex interactions among yeasts in synergistic relationships are not well known, but may be involved in the enhanced pathogenic potential of these associations. In this investigation, mixed-species were reduced by 50% and 54% in the NYT and PDT groups, respectively, at the end of the treatments. This suggests that both treatments were effective in reducing more complex biofilms.

In conclusion, despite the limitations of this first clinical investigation, such as the low number of patients evaluated, the results demonstrated that PDT was as effective as topical nystatin in reducing the CFU/mL of *Candida* spp. from the dentures and palates of patients with DS, but a higher number of patients showed decrease in palatal inflammation after nystatin. In addition, a high rate of recurrence was observed, and *C. albicans* was the most prevalent species identified. Further studies are required to determine more effective clinical parameters for a better response to PDT in patients with DS, and more patients should be assessed to draw firmer conclusions. The principal advantage of PDT is that, unlike antifungal agents, development of resistance to phototherapy seems to be improbable because of its mechanism of action, and potential toxic or adverse effects of PDT on palatal tissue are unlikely. Moreover, according to the results of this investigation, fewer sessions of PDT are necessary to achieve the same clinical outcome as nystatin, which, on the other hand, requires multiple daily doses, which can lower the patient's compliance. As PDT is performed in the dental office, dentists can also monitor the therapeutic sessions and the patient's response gradually. Therefore, PDT seems to be a promising method for the treatment of DS.

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Transparency Declaration

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References

1. Barbeau J, Séguin J, Goulet JP *et al.* Reassessing the presence of *Candida albicans* in denture-related stomatitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; 95: 51–59.

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2. Figueiral MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C. Denture-related stomatitis: identification of aetiological and predisposing factors – a large cohort. *J Oral Rehabil* 2007; 34: 448–455.
3. Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol* 2008; 23: 377–383.
4. Cross LJ, Williams DW, Sweeney CP, Jackson MS, Lewis MA, Bagg J. Evaluation of the recurrence of denture stomatitis and *Candida* colonization in a small group of patients who received itraconazole. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004; 97: 351–358.
5. Mima EG, Pavarina AC, Silva MM *et al.* Denture stomatitis treated with photodynamic therapy: five cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 112: 602–608.
6. Pires FR, Santos EB, Bonan PR, De Almeida OP, Lopes MA. Denture stomatitis and salivary *Candida* in Brazilian edentulous patients. *J Oral Rehabil* 2002; 29: 1115–1119.
7. Sanitá PV, Pavarina AC, Giampaolo ET *et al. Candida* spp. prevalence in well controlled type 2 diabetic patients with denture stomatitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 111: 726–733.
8. Zomorodian K, Haghighi NN, Rajaee N *et al.* Assessment of *Candida* species colonization and denture-related stomatitis in complete denture wearers. *Med Mycol* 2011; 49: 208–211.
9. Shapiro RS, Robbins N, Cowen LE. Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiol Mol Biol Rev* 2011; 75: 213–267.
10. Chandra J, Mukherjee PK, Leidich SD *et al.* Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. *J Dent Res* 2001; 80: 903–908.
11. Perea S, Patterson TF. Antifungal resistance in pathogenic fungi. *Clin Infect Dis* 2002; 35: 1073–1080.
12. Banting DW, Greenhorn PA, McMinn JG. Effectiveness of a topical antifungal regimen for the treatment of oral candidiasis in older, chronically ill, institutionalized, adults. *J Can Dent Assoc* 1995; 61: 199–200, 203–205.
13. Pallasch TJ. Antifungal and antiviral chemotherapy. *Periodontol 2000* 2002; 28: 240–255.
14. Donnelly RF, McCarron PA, Tunney MM. Antifungal photodynamic therapy. *Microbiol Res* 2008; 163: 1–12.
15. Konopka K, Goslinski T. Photodynamic therapy in dentistry. *J Dent Res* 2007; 86: 694–707. Erratum in: *J Dent Res* 86: 1126.
16. Mima EG, Pavarina AC, Dovigo LN *et al.* Susceptibility of *Candida albicans* to photodynamic therapy in a murine model of oral candidosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 109: 392–401.
17. Trindade FZ, Pavarina AC, Ribeiro AP, Bagnato VS, Vergani CE, Costa CA. Toxicity of photodynamic therapy with LED associated to Photogem®: an *in vivo* study. *Lasers Med Sci* 2012; 27: 403–411.
18. Donnelly RF, McCarron PA, Tunney MM, David Woolfson A. Potential of photodynamic therapy in treatment of fungal infections of the mouth design and characterisation of a mucoadhesive patch containing toluidine blue O. *J Photochem Photobiol, B* 2007; 86: 59–69.
19. Dovigo LN, Pavarina AC, Mima EG, Giampaolo ET, Vergani CE, Bagnato VS. Fungicidal effect of photodynamic therapy against fluconazole-resistant *Candida albicans* and *Candida glabrata*. *Mycoses* 2011; 54: 123–130.
20. Teichert MC, Jones JW, Usacheva MN, Biel MA. Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; 93: 155–160.
21. Mima EG, Pavarina AC, Ribeiro DG, Dovigo LN, Vergani CE, Bagnato VS. Effectiveness of photodynamic therapy for the inactivation of *Candida* spp. on dentures: *in vitro* study. *Photomed Laser Surg* 2011; 29: 827–833.
22. Ribeiro DG, Pavarina AC, Dovigo LN *et al.* Photodynamic inactivation of microorganisms present on complete dentures a clinical investigation: photodynamic disinfection of complete dentures. *Lasers Med Sci* 2012; 27: 161–168.
23. Newton AV. Denture sore mouth as possible aetiology. *Br Dent J* 1962; 112: 357–360.
24. Barnabé W, de Mendonça Neto T, Pimenta FC, Pegoraro LF, Scolaro JM. Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, *Streptococcus mutans* and *Candida albicans*. *J Oral Rehabil* 2004; 31: 453–459.
25. Neppelenbroek KH, Pavarina AC, Palomari Spolidorio DM, Sgavioli Massucato EM, Spolidorio LC, Vergani CE. Effectiveness of microwave disinfection of complete dentures on the treatment of *Candida*-related denture stomatitis. *J Oral Rehabil* 2008; 35: 836–846.
26. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159–174.
27. Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun* 1985; 50: 97–101.
28. Vanden Abbeele A, de Meel H, Ahariz M, Perraudin JP, Beyer I, Courtois P. Denture contamination by yeasts in the elderly. *Gerodontology* 2008; 25: 222–228.
29. Jayatilake JA, Samaranayake YH, Samaranayake LP. An ultrastructural and a cytochemical study of candidal invasion of reconstituted human oral epithelium. *J Oral Pathol Med* 2005; 34: 240–246.

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