Photodiagnosis and Photodynamic Therapy 28 (2019) 98–101

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Efficacy of photodynamic therapy versus local nystatin in the treatment of denture stomatitis: A randomized clinical study

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ARTICLE INFO

Keywords:
Candida albicans
Photodynamic therapy
Nystatin
Clinical trial
Antimicrobial photodynamic therapy
Photodynamic inactivation

ABSTRACT

Aim: The aim of the present randomized clinical study was to compare the efficacies of photodynamic inactivation (PDI) to nystatin (NST) in terms of prevalence of *Candida* species in cases with denture stomatitis (DS).
Methods: Thirty-six patients were randomly divided into two groups; 18 in PDI and 18 in NST. Irradiation was carried out by using the GaA1As diode laser with wavelength, mode of transmission, laser output and energy density were standardized at 660 nm, continuous mode, 100 mW power and 28 J/cm2 respectively. The PDI was applied twice a week, with an interval of at least 48 h among the sessions during four weeks. Topical nystatin oral suspension 100,000 IU was used four times daily for 15 days. The existence of *Candida* spp. was confirmed by employing the microbiological culture technique. *Candida* colony counts from the palates and dentures surfaces, quantified as colony forming unit (CFU)/mL, measured at baseline, at the end of treatments (day 15), and at follow-up (days 30 and 60) and the prevalence of *Candida* spp. were identified in the two groups of treatments.
Results: The overall CFU/mL values were higher in the dentures of the patients of both the groups than those from the palates. During all time periods of the study, the CFU/mL values obtained from both NST and PDI groups showed no significant differences. For dentures and palates, a significant reduction in mean CFU/mL values was observed on day 15 compared with baseline (day 0) in both NST and PDI groups. It can be seen that the effect size of treatments was large for the palates of patients in the NST group (1.79) and moderate for the palates of patients in the PDI group (0.63). On the other hand, the effect size was very large for the dentures for both groups (NST group = 3.01; PDI group = 1.58). *C. albicans* was the most common species on both dentures and palates of patients throughout the study period followed by *C. tropicalis* and *C. glabrata*.
Conclusion: Out of all the *Candida* spp., *C. albicans* showed the highest prevalence among all species. In addition, PDI was equally effective as nystatin for the treatment of DS.

1. Introduction

Denture stomatitis (DS) is an extremely common condition experienced by denture wearers. Several studies have stated that at least one-third or more individuals who wear removable complete dentures are susceptible to this condition [1,2]. This inflammatory condition is described by varying grades of mucosal inflammation underneath maxillary dentures, displaying petechiae formation to generalized papillary hyperplasia [3]. DS is a multifactorial condition and the causes usually include decreased salivary output, endocrinopathies, metabolic factors, medication, smoking, nutritional factors, poor adaptation of dentures, uninterrupted denture wearing and compromised dental and denture hygiene respectively [4]. Fungal (primarily *Candida*) and bacterial infections also play a role in the establishment of DS [5].

Candida albicans is the most frequently found *Candida* spp. that is associated and significantly isolated with DS [6,7] This specie of *Candida* cultivates as a biofilm at the hard and soft tissue of the oral cavity. The biofilm of *Candida* is a complex matrix of extracellular polysaccharide, which allows it to show increased resistance to antimicrobials [8]. With *C. albicans* being highly threatening of the

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https://doi.org/10.1016/j.pdpdt.2019.08.028
Received 3 August 2019; Received in revised form 17 August 2019; Accepted 23 August 2019
Available online 24 August 2019

characteristic genus *Candida*, other non-*Candida* species like *C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. pseudotropicalis and C. guilliermondii* are also found on the palatal mucosa and acrylic surface of the denture. [9] These species may also play a role in the onset of DS infection [10].

The treatment of DS can easily be done with the help of antifungal agents, in correspondence with optimum maintainence of the denture, reduced nocturnal wear and proper oral hygiene measures [11,12]. Miconazole, nystatin (NST) and other topical agents can be used for the treatment but the cleansing activity of the oral musculature reduces the therapeutic level. Whereas, systemic antifungals like Amphotericin B can be used, but they are not very effective in removing the fungal colonies that occupy the surface of the denture [13].

Because of the development of the species having aggressive resistance against the anti-fungal drugs, new and innovative ideas need to be employed for establishing better treatment modalities. Photodynamic inactivation (PDI), also called antimicrobial photodynamic therapy, is a relatively new treatment modality for the eradication of fungal activity. In this method, the energy of the light source is absorbed by the photosensitizer (PS), where the PS further transfers the energy to oxygen, resulting in the production of harmful oxygen species. These species destroy the microorganism via oxidative stress [14,15] and is useful in the treatment of various oral diseases including periodontal diseases, peri-implant diseases, lichen planus, and various fungal infections [16–23]. To date, no study have been performed that evaluated the anti-fungal efficacy of methylene blue (MB) mediated PDI and compared it with oral suspension NST in the treatment of DS. Therefore, the aim of the present randomized clinical study was to compare the efficacies of PDI with NST in terms of prevalence of *Candida* species in cases with DS.

2. Methodology

2.1. Ethics

Ethics research approval was acquired from ethics research committee of Centre for specialist dental practice and clinical research (UDCRC/077-93221). All participants included in the research obtained written and verbal informed consents with the signatures. They were granted the right to pull out from the research study anytime.

2.2. Selection

For inclusion criteria, patients with an age ranging from 40 to 65 years, having removable complete dentures diagnosed with DS (sensation of mucosal burning, erythema and loss of filiform papillae) were selected [24]. Exclusion criteria included the detection of any systemic ailments like diabetes, heart problems, cancer or acquired immune deficiency syndrome (AIDS). Gravid females, illness during the treatment routine, history of any use of antimicrobial therapy in the past two months and failing to attend to more than one clinical therapy sessions were also not included in the present study.

2.3. Sampling of Candida spp

Candida spp. were confirmed by employing the microbiological culture technique. For this purpose, the material was obtained from the maxillary complete denture prosthesis and the palatal mucosa. Swabs were deposited in a test tube with predetermined 5 mL of 0.9% sterile saline and vortexed for 60 s so that the organisms are suspended from the swab. For the colony counting and the identification of species of *Candida*, the swabs used to obtain the material from the designated site underwent the process of incubation at 37C for 24 h. Primary culture medium CHROMagar *Candida* was used for the probable documentation of species and general characteristics including the texture, colour and morphology of the species were also evaluated. Moreover, the colonies of *Candida* spp were stained by the Gram method.

2.4. Randomization and treatment protocol

In order to evaluate the antifungal effect of PDI and NST on the denture prosthesis and *Candida* spp, the subjects underwent block randomization and were divided into two separate groups of 18 patients each. For randomization, every block consisted of 6 patients (3 blocks per group) that recruited patients after clinically examining signs for DS and on the basis of convenience sampling. Assessors providing therapy were blinded to the study groups. Codes were revealed after providing the treatment and assessments. Patients were instructed to scrub their dentures using a toothbrush and toothpaste after meals and before bed time. Patients were also taught how to soak dentures overnight in filtered water. At the day of treatment, all dentures were again bathed with water and left to dry on absorbent paper.

2.5. PDI group

Eighteen patients were assigned to the PDI group after randomization. Both the surfaces of the denture and palatal mucosa were sprayed with MB photosensitizer having a concentration of 450 μg/mL for 10 min of pre-irradiation time. Irradiation was carried out by using the gallium-aluminum-arsenium (GaA1As) diode laser by thoroughly scanning the anatomical structural of palatal mucosa and the removable maxillary denture prosthesis. Moreover, the characteristics of the laser including the wavelength, mode of transmission, laser output and energy density were standardized at 660 nm (nm), continuous mode, 100 mW (mW) power and 28 J/cm^2, respectively. The PDI was carried out twice in one week, each session with a gap of 48 h and during four weeks.

2.6. NST group

In the NYT group, a total of 18 patients were assigned after randomization. In these patients, the local antifungal treatment was done with oral suspension of 100,000 IU nystatin. On receiving the drug, each patient was trained on how to use the medication, that is, gargling for 60 s and expectorate. This was carried out daily four times for 2 weeks.

2.7. Outcomes

Primary outcome of the study was the *Candida* counts from the palates and surfaces of dentures, estimated as colony forming unit per millilitre (CFU/mL). These counts were measured at baseline, day 15 (end of treatments), and at follow-up (days 30 and 60), whereas the secondary outcome was the total percentage counts of *Candida* spp. detected in both treatment groups.

2.8. Statistical analysis

Normal distribution of data was verified using the Shapiro-Wilk test was employed. For the microbiological analysis of mucosa and prosthesis, the Wilcoxin test was used to check the comparison within the groups, whereas the Fisher's exact test was used to establish a comparison between the groups of interest. Statistically significant differences were established at $p < 0.05$.

3. Results

From Table 1, it can be observed that the overall CFU/mL values were higher in the dentures of the patients of both the groups than those from the palates. During all time periods of the study, the CFU/mL values obtained from both NST and PDI groups showed no significant differences. The recordings from therapeutic groups were

Table 1
Means Candidal counts in CFU/mL found on palates and dentures following two treatment modalities.

Group	Location	Follow-up				
		Day – 0	Day 15	Day 30	Day 60	Effect size
NST	Palate	5.83 ± 3.31	1.87 ± 2.05*	2.04 ± 3.98*	3.16 ± 3.79*,‡	1.79
	Denture	33.76 ± 8.80	14.86 ± 16.32*	22.49 ± 18.74*,‡	24.56 ± 20.21*,‡	3.01
PDI	Palate	4.92 ± 2.13	3.72 ± 4.12*	3.96 ± 4.13	3.28 ± 4.93‡	0.63
	Denture	36.47 ± 10.94	20.57 ± 21.83*	35.38 ± 17.84‡	33.71 ± 21.66‡	1.58

* Significantly different from baseline ($p < 0.05$).
‡ Significantly different from day 15 ($p < 0.05$).

assessed at the same time of recording days (baseline, day 15, day 30 and day 60). A statistically significant reduction in mean CFU/mL values was noted on day 15 compared with baseline in both NST and PDI groups on both palates and dentures. At days 30 and 60, a significant reduction in *Candida* spp was observed on both palates and dentures in the NST group only ($p < 0.05$). The decline was much higher on day 15 compared to the days 30 and 60, respectively. In the PDI group, there was a statistically significant reduction in the CFU/mL values on both palates and dentures at 60 day follow-up compared with the end of day 15 therapy. There was a statistically significant reduction only on dentures that was observed on day 30 compared with day 15 treatment. It is note-worthy that considerable therapeutic effect size was noted for the palates of patients in the NST group (1.79) and average for the palates of patients in the PDI group (0.63). On the other hand, the effect size was significantly large for the dentures for both groups (NST group = 3.01; PDI group = 1.58).

It can be observed from Table 2 that *C. albicans,* out of all the species, were dominantly found on both dentures and palates of patients throughout the study period. After that, *C. tropicalis* showed the second highest prevalence, followed by third most common species included *C. glabrata*. Additional *Candida* spp were found less prevalent. By the end of day 15 compared with the baseline data, the number of *Candida* spp including *C. albicans*, *C. tropicalis* and *C. glabrata* significantly dropped down by 51%, 25% and 28.5%, respectively in the NST groups, and 51.8%, 50% and 20%, respectively in the PDI group. These species increased compared to day 15 treatment at day 30 and day 60. The combination of two *Candida* spp. including *C. albicans* and *C. tropicalis* were the highest in both NST and PDI groups.

4. Discussion

The aim of the present randomized clinical study was to evaluate the prevalence of *Candida* spp. before and after two therapeutic modalities including PDI and NST in the treatment of denture stomatitis. The results of this clinical trial revealed that *C. albicans* showed the highest dominance of all the yeast in terms of prevalence. Moreover, PDI showed comparable therapeutic efficacy compared with NST in the treatment of DS. The findings of the present study corroborates with the results from previous clinical studies that showed thrice application of light emitting diode light significantly reduced *Candida* colonies with clinical success rate of 45% compared to 53% with NST therapy [25]. In another case study of 5 patients revealed that PDI helped in resolving clinical inflammation in patients with DS.

Table 2
Number of Candidal spp on dentures and palates found among NST and PDI groups at follow-up.

Species	Day – 0		Day – 15		Day – 30		Day – 60		Total	
	NST	PDI	NST	PDI	NST	PDI	NST	PDI	NST	PDT
C. albicans	32	27	17	14	19	20	24	19	92	80
C. tropicalis	8	10	2	5	6	4	7	9	23	28
C. glabrata	7	5	2	1	5	2	5	4	19	12
C. krusei	2	1	0	0	0	0	0	0	2	1
C. lusitaniae	1	0	0	0	0	0	0	1	1	1
C. parapsilosis	3	1	0	0	0	0	0	0	3	1

It is note-worthy that the follow-up period showed a significantly higher candidal counts compared to the end of the treatment at 15 day. This outcome was inevitable as *Candida* and other microbial species have a high affinity of progressive recolonization on hard surfaces. This is one of the main virulence factor including cell-surface hydrophobicity and ability to multiply and form biofilms augments the adherence to acrylic denture surfaces [26,27]. Research studies have also revealed that *Candidal* carriage is substantially found on the tissue-interface of acrylic dentures rather than on the soft tissue of palate, with or without clinical inflammation on the oral mucosa [28]. This was never confirmed through palatal cultures due to the sensitive techniques involved for collection of samples (for instance swab technique) which leads to low level of cultures in consecutive follow-up periods. In an in-vitro analysis concluded that *C. albicans* was able to ingress a reconstituted human oral epithelial surface over a period of 2 days through hyphal penetration [29]. Therefore, other non-sensitive sampling techniques may help to evaluate the overall fungal burden on the oral mucosa of patients at baseline and following therapy.

The current study suggested a greater level of clinical outcome that was confirmed in the NST group compared to the PDI group. Antifungal would have also decreased yeast cells on the soft tissue surfaces including buccal mucosa and dorsum of the tongue from the anti-fungal group, which could validate the increased rate of resolution in NST group. It should also be noted that no antibacterial therapy of the dentures was carried out in this group that may have added to the low clinical success in this group. The low clinical success detected among patients in PDI group could be due to the recolonization by *Candida* spp on the palatal mucosa after laser therapy from adjacent areas of the mouth. Moreover, the conditions in which photodynamic therapy was performed, such as treatment time (carried out twice in one week, each session with a gap of 48 h and during four weeks) and laser parameters (only one photosensitizer type and its concentration and energy fluence) may also suggest the low level of therapeutic success noted in the PDI group. Long follow-up duration with more clinical sessions of PDI could attain improved recovery of palatal inflammation. More clinical studies are required in order to assess the impact of other laser related parameters of PDI, including the different concentrations as well as the types of photosensitizers with other parameters such as laser fluences, in an effort to achieve increased clinical success.

The findings of the current study demonstrated that *C. albicans* was the highest obtained from both palates and denture surfaces among both treatment groups. These results are also in agreement with previous studies [6,7,30]. Following these, the second most prevalent organisms observed were *C. tropicalis* and then followed by *C. glabrata* on dentures and palates. It is well-established that *C. tropicalis* is generally the second highest microorganisms separated from oral environment [6,31]. On the contrary, other researchers have observed that *C. glabrata* was the most common yeast after *C. albicans* [4,30]. In our study, substantial reduction of yeasts were verified at the end of the treatments. This shows that these yeasts were predisposed to both treatment

modalities, except *C. tropicalis*, that resulted in higher vulnerability to nystatin than PDI. It may be speculated that these augmented species found in the successive follow-up periods when compared with day-15 therapy may suggest proliferation and multiplication over the soft tissues and denture surfaces.

The present study have some limitations that should be considered. The current study followed up for a short period of time. In addition the current study only relied on rehabilitation of subclinical inflammation in the oral cavities of the patients. Interventions involving resolution of inflammation on cellular levels may have attributed to far more specific therapeutic outcomes. Furthermore, the most important limitation is the cost of the treatment involved. Laser therapies usually involve a higher cost and is generally non-affordable for patients.

5. Conclusion

Out of all the *Candida* spp., *C. albicans* showed the highest prevalence among all species. In addition, PDI was equally effective as nystatin for the treatment of DS.

Declaration of Competing Interest

The authors declare no conflict of interest in the present study.

Acknowledgement

The authors are grateful to the Deanship of Scientific Research, King Saud Universityfor funding through Vice Deanship of Scientific Research Chairs, Research Chair for Biological Research in Dental Health.

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