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Research Paper

Evaluation of photodynamic therapy efficacy vs. conventional antifungal therapy in patients with poor-fitting dentures suffering from denture stomatitis. A prospective clinical study

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ABSTRACT

Background: The long-term use of antifungal therapy in denture stomatitis (DS) treatment could be accompanied by antifungal-resistant strain onset, leading to compromised therapeutic procedure and disease reappearance. Photodynamic therapy (PDT) has shown the ability to eradicate oral infections and resistance strains. This prospective clinical study aimed to assess the PDT's effectiveness compared to the conventional treatment on clinical and microbiological parameters in patients with DS without denture wear during the treatment and follow-ups.

Methods: Forty-two patients diagnosed with DS were randomly assigned to one-session single PDT application (test group) or conventional antifungal therapy (control group). Clinical and microbiological parameters were assessed and analyzed before and at 3rd, 15th, and 30th day following the treatments. Microbiological samples were analyzed by a Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The data was statistically analyzed.

Results: Prior to the treatment, *Candida* species, including *C. albicans* (100%), *C. glabrata* (33%), *C. tropicalis* (31%), *C. krusei* (31%) were isolated in all patients. Both treatment procedures demonstrated a statistically significant reduction in C. albicans at all follow-up time intervals ($p < 0.05$). However, PDT displayed a statistically significant reduction in C. krusei compared to the conventional treatment at all follow-up periods ($p < 0.05$). Clinical parameters improved considerably in the test group compared to the control group at the 3rd and 15th day of follow-up.

Conclusion: One-session single PDT application demonstrated significant improvement in both clinical and microbiological outcomes in a short-term period, resulting in complete Candida spp. eradication compared to conventional antifungal therapy.

1. Introduction

Infection caused by fungi is considered as one of the foremost issues in public health, being diagnosed in millions of people annually [1]. The oral cavity represents a common site for fungal infection establishment and development. In majority of cases, fungal infection may be detected in immunocompromised patients or newborns with severe systemic diseases, patients who had undergone radiotherapy, patients who had received long-term antibiotic therapy as well as partially or totally edentulous patients with removable dentures [2,3].

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Chronic atrophic candidiasis or denture stomatitis (DS) has been known as very common oral cavity disease that affects around 70% of patients with removable complete or partial acrylic dentures [4,5]. Denture material characteristics such as porosity and roughness have the ability to provide an anaerobic and acidic ecological niche in which microorganisms, including *Candida* species [6] and bacteria (*Streptococci, Fusobacteria,* and *Bacteroides)* could colonize, grow, and multiply, respectively [7,8]. DS can be caused by *Candida albicans,* which is an opportunistic microbe of the oral cavity. However, in acidic conditions, *C. albicans* can transform into a pathogen, changing the morphological forms from blastophore to hyphae and penetrating deeper tissue layers. Furthermore, other species of the *Candida* genus such as *C. glabrata, C. tropicalis, C. parapsilosis, C. pseudotropicalis, C. krusei, and C. guilliermondii* [9] were isolated from acrylic removable restorations and the palate, [10] and might contribute to disease development. In addition to microbes, numerous etiological and risk factors, including poor oral hygiene associated with poor-fitting dentures, nocturnal denture wearing, smoking, reduced saliva flow, and xerostomia, facilitate denture stomatitis onset [5,11,12]. These factors can altogether lead to various grades of oral mucosa inflammation from petechiae to generalized inflammation and hyperplasia [13]. Although DS is an asymptomatic disease, it has been noted that some cases with sensations and symptoms such as burning, dysgeusia, dysphagia, and halitosis [11] can significantly impair the patients' quality of life [14].

Denture stomatitis requires a multidisciplinary approach which includes not only antifungal therapy in gels, creams, or oral suspensions but also identification and correction of etiological and risk factors and proper oral hygiene maintenance [15]. It has been shown that several species, including *C. glabrata* and *C. krusei*, can decrease treatment efficacy, and thus are substantial for detection. Antifungal therapy administered topically often involves repeated treatment on a long-term basis, which could contribute to resistant strain development and the systemic antifungal drug prescription, respectively [16]. Unfortunately, systemic antifungal drugs, such as amphotericin B and fluconazole, have shown to be ineffective in *Candida* colonies eradication from the palate [16], hence resulting in treatment failure, disease recurrence, and impaired quality of life. Furthermore, it is essential to find alternatives to conventional antifungal therapy so as to eradicate resistant strains and persistent infections while simultaneously decreasing treatment time.

As a non-invasive approach, photodynamic therapy (PDT) appears to be highly effective in the treatment of various oral infections, particularly fungal and bacterial ones [17]. The PDT working mechanism is based on the photochemical reaction in which highly reactive oxygen species (ROS), including single oxygen, are produced in the presense of tissue oxygen, causing pathogen death. Thus, this treatment modality has been proposed as a beneficial approach for numerous oral diseases, including periodontitis [18–20], peri-implant disease [20–22], chronic periapical periodontitis [23–26], and oral candidiasis [27–32]. Furthermore, in oral candidiasis treatment, ROS generated by the PDT [33] turned out to be effective at removing fungus-resistant stains [34]. As a result, PDT has shown efficient elimination of the *Candida* spp. completely or partially in several experiments and animal studies [28, 30,34-36]. Despite the fact that clinical studies have demonstrated substantial *Candida* spp. eradication from the palate and dentures [29, 34], more clinical studies found no difference between PDT and antifungal therapy [31]. Additionally, reappearance and recurrence of fungal infection was detected after 30 to 45 days following PDT [27,37]. Subsequently, a lack of treatment protocol standardization [34], the number of PDT applications, different pre-irradiation and irradiation time, and ongoing full-time denture wear during and after the treatment could be considered as reasons for inconsistent results. Considering the structure of removable acrylic dentures, which are said to be difficult to completely eradicate microbes from, the question arises whether these dentures have to be worn during the therapy and the follow-ups and whether new dentures are required to be made following the treatment.

The aim of this study was to assess the clinical and microbiological outcomes after a one-session single application PDT compared to conventional antifungal therapy in patients without denture wearing during the treatment and follow-ups. A hypothesis was that a single one-session PDT application could produce significant improvements in clinical and microbiological outcomes compared to conventional antifungal therapy.

2. Material and method

2.1. Study design and population

The study was conducted as a prospective comparative study approved by the Ethics Committee (No. 36/27), School of Dental Medicine, University of Belgrade, Serbia, and in accordance with the Declaration of Helsinki of 1975, revised in 2008. Informed consent was obtained from all participants prior to the study.

Patients with complete or partial acrylic dentures in the maxilla or mandible were evaluated and recruited at Department of Prosthodontics and Department of Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade, between January 2021 and April 2023. For further examination, only patients with DS clinical features such as oral mucosal inflammation accompanied by erythema (Fig. 1a) with/without hyperplasia (Fig. 2a) and poor-fitted dentures that were required to be changed or relined were recruited. Furthermore, to be eligible for the enrollment in the study, patients had to be over 35 years old with signed informed consent and had isolated *Candida* spp. (sample 1).

DS with non-detection of *Candida* spp., patients with diabetes mellitus, anemia, gastroesophageal reflux, immunocompromised patients and on immunosuppressive medications (radiotherapy or chemotherapy), history of antimicrobial use in the last two months, and pregnancy and lactation were excluded criteria.

2.2. Microbiological sampling and analyses

The isolation, identification, and semiquantification of *Candida* spp. were determined by swab sampling from the palate or alveolar ridge of oral mucosa. Swabs were collected before the treatment procedure (sample 1), on the 3rd (sample 2), 15th (sample 3), and 30th days following the therapies (sample 4). Each swab was placed into a transport medium (eSwab LQ Amies, COPAN Diagnostics Inc., USA) and was transported to the Institute for Microbiology, Medical Military Academy, Belgrade, Serbia.

The samples were inoculated according to standard procedures for fungi identification established at the Institute for Microbiology, Medical Military Academy, Belgrade, Serbia. Briefly, the swab within the transport medium was vortexed, and the 200 μl of transport medium was inoculated on the Sabouraud Dextrose Agar (SDA, HiMedia, India), respectively. Subsequently, cultivated, and isolated colonies were incubated at 36.0 °C for 24 h before being identified. The colonies were identified using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF mass spectrometry "VITEK MS", bioMerieux, France). A semi-quantification assessment was conducted using sterile inoculating loops in all directions on the selective medium. Each dilution step was rated as *low* if only a colony was detected, *medium* if two to ten colonies were detected, and *abundant* if more than ten colonies were detected.

2.3. Data collection

Data collection included demographic information and risk factors such as smoking (smoker > 10 cigarettes/day, and non-smoker) and oral hygiene habits, as well as denture assessment and maintenance, and clinical examination.

Regarding denture assessment, the following parameters were considered:

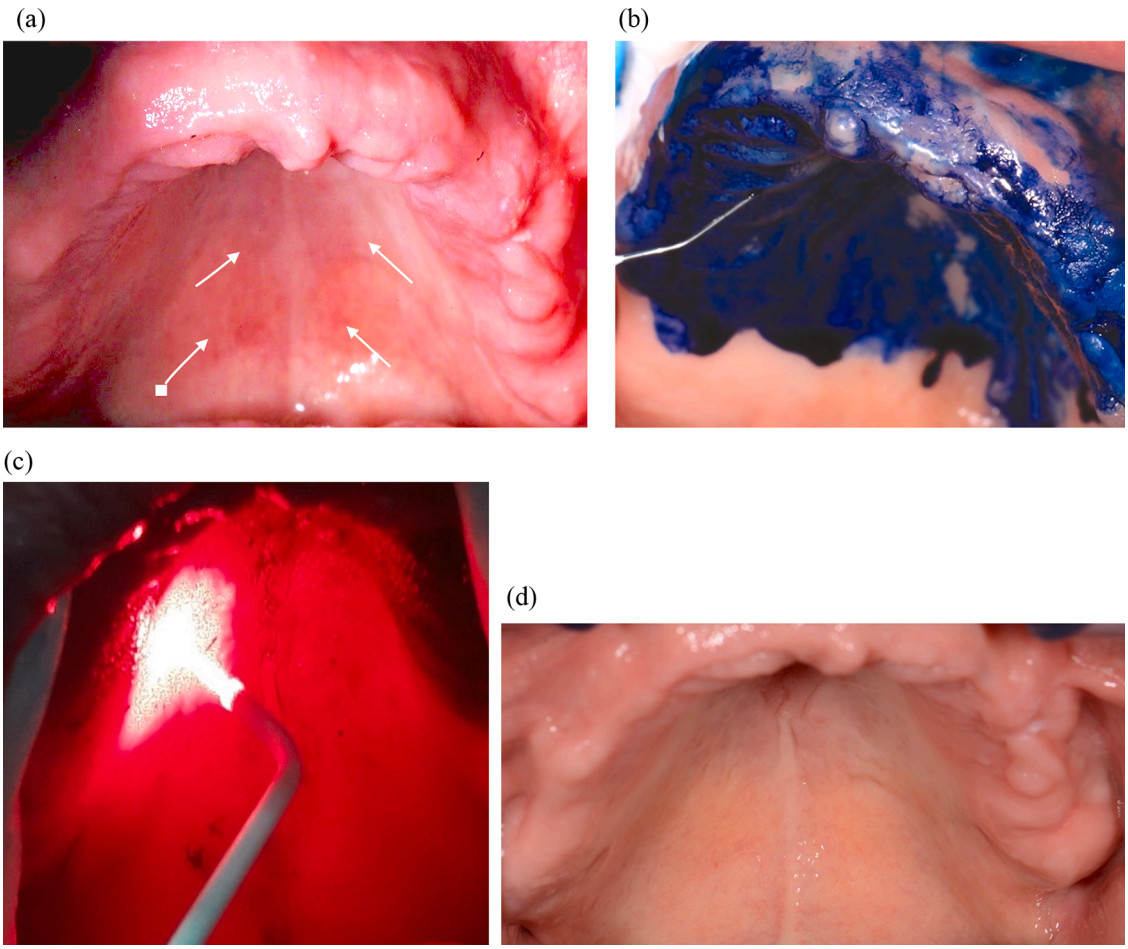

Fig. 1. Photodynamic therapy application in the patient with denture stomatitis Newton type I before treatment (a), during the therapy (b, c), 15th day after the treatment (d). The area of denture stomatitis prior the treatment was represented with white arrows.

1. Materials from which the denture was made.
2. History of denture relining: Yes/No.
3. Denture oral hygiene maintenance methods.
4. Denture wearing frequency: a. Nocturnal denture wearing; b. Taking off for a couple of hours twice a week; c. Never taking the denture off.

The clinical efficacy of the treatment procedures was determined by estimating the clinical response of oral mucosa and assessment of the changes in clinical parameters in the palatal mucosa and alveolar ridge mucosa in both maxillae and mandible. The clinical parameters were based on the Newton classification [38] with respect to oral mucosa inflammation, scored by two experienced dentists (J.M., E.J.), blinded to the treatment procedures as 0- no inflammation, 1- localized erythema, 2- generalized erythema, and 3- localized/generalized erythema with hyperplasia. A score was determined before the treatment as well as at 3rd, 15th, and 30th day after the treatment. Additionally, the standardized photographs were taken before the treatments and at follow-up periods for assessing the clinical response of the oral mucosa.

2.4. Treatment

Prior to the treatment approach a white envelope was used for randomization, and the patients were randomly assigned into two groups: test (PDT) and control (C). In both groups, the patients were asked not to wear their dentures during the treatment intervals and follow-up appointments. Additionally, patients were instructed to maintain proper oral hygiene and not to use additional treatment methods such as baking soda or chlorhexidine di-gluconate.

In the test group, patients were treated with a one-session single application of photodynamic therapy (PDT, Fig. 1b, c, and 2b). Briefly, a photosensitizer, phenothiazine-derivate methylene blue (3,7-bis(dimethylamino)-phenothiazinium chloride, concentration of 9 mg/ml), was applied onto the palate and alveolar ridge of the maxilla/mandible for 5 min and irrigated with saline solution. Subsequently, the palate mucosa and alveolar ridge were irradiated by means of a fiber optic tip (0.05 cm^2 size, 12 mW power, HELBO® 3D Pocket Probe) and diode laser (HELBO® TheraLite Laser; Photodynamic Systems GmbH, $\lambda = 660$ nm, power = 100 mW) on continuous mode for 30 s/ point. The total energy dose was 180 J/ cm^2. A total exposure time of 5 to 8 min was determined based on the size of the irradiated area.

In the control group, patients were instructed to take topical antifungal therapy- nystatin oral suspension (NYS, 100,000 IU), four times a day for 14 days. Patients with denture in the antagonist dental arch were instructed to remove the denture before treatment and brush their teeth and the denture.

In cases of *Candida* reappearance or incomplete eradication after 30 days of follow-up, patients were advised to have additional conventional topical and systemic antifungal therapy.

After completing the follow-up time intervals, patients were referred to the Department of Prosthodontics to assess the possibility of making a new denture or relining the existing one. Denture relining was performed on dentures that were fabricated less than 3 years and were displaying minimal movements during function. Prior to relining, dentures were brushed, decontaminated in an ultrasonic bath, and superficial layer was removed. Afterward, the impression was obtained, and

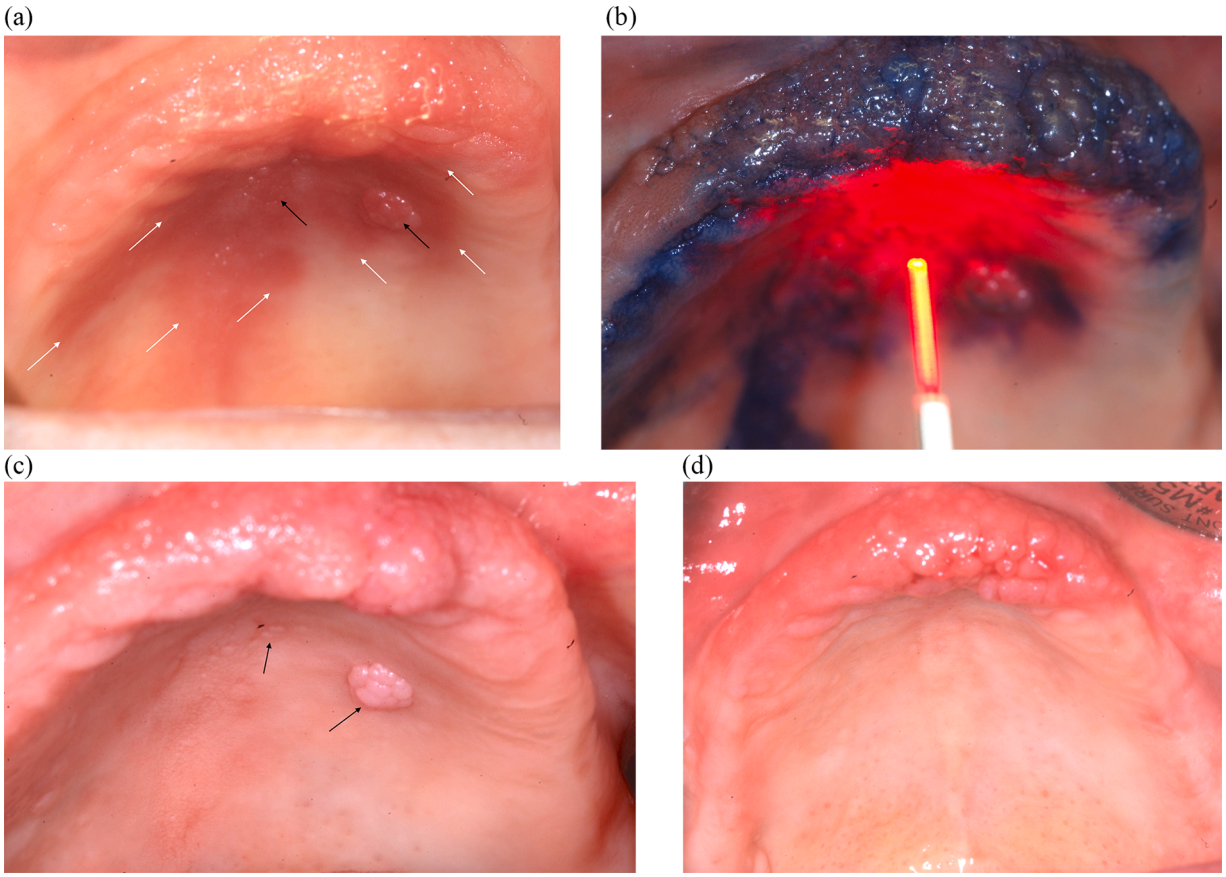

Fig. 2. Photodynamic therapy treatment in patient with denture stomatitis, defined as Newton-type III, before treatment (a), during the therapy (b), 3rd (c), and 15th day after the treatment (d). Denture stomatitis was accompanied by inflammation of the palatal oral mucosa and/or alveolar region including erythema (white arrows) and hyperplasia (black arrows) before treatment (a). On the 3rd day, the erythema was absent, but hyperplasia remained (c). On the 15th day, there were no signs of hyperplasia or erythema on the palatal oral mucosa (d).

the relining was carried out in a dental laboratory.

2.5. Sample size and statistical analyses

Considering the effect size of photodynamic therapy compared with conventional antifungal therapy in the study of Mima et al. [27], the sample size calculation was conducted by using G*Power software version 3.1. With a power of 80% and $\alpha = 0.05$, assuming that an SD would not exceed 10%, a total of 14 patients were needed per treatment group. Due to possible dropouts, 17 patients who met inclusion criteria were recruited.

The data was analyzed using Prism version 10 (GraphPad Software). Results were presented as counts (%) or means ± SD, depending on the data type. Data distribution was evaluated with the Shapiro-Wilk test. Differences between groups were assessed using the Mann-Whitney test or Chi-square test. The differences in the presence of *Candida* spp. for different treatments were assessed using 2-way ANOVA. All p - values less than 0.05 were considered significant.

3. Results

The study involved 42 patients homogeneously assigned to two groups (Table 1), with mean ages of 62 ± 11 (test group), and 62 ± 6 (control group). In terms of ages and gender, no statistically significant differences were found between the groups. In the study, more than one-third of the patients were smokers (> 10 cigarettes/ day). There were statistically significant differences between non-smokers and smokers within groups ($p = 0.031$). The smokers were not homogenously distributed between the groups ($p = 0.033$).

Table 1
Socio-demographic and risk factors of the patients with diagnosed denture stomatitis at baseline*.

		Test group ($n = 20$)	Control group ($n = 22$)	p - value
Ages, mean ± SD		62 ± 11	62 ± 6	0.693
Gender, n (%)	Male	6 (30%)	10 (45%)	0.695
	Female	14 (70%)	12 (55%)	0.317
Smoker habits, n (%)	Smoker	3 (15)	11 (50)	0.033*
	Non-smoker	17 (85)	11 (50)	0.257
Systemic diseases, n (%)	Health condition	10 (50)	8 (36)	0.637
	HTA	8 (40)	11 (50)	
	OT CVD	0 (0)	3 (14)	
	HTy	2 (10)	0 (0)	
Location, n (%)	Maxillae	18 (90)	16 (70)	0.580
	Mandible	2 (10)	6 (30)	0.480
Newton classification, n (%)	Class I	2 (10)	0 (0)	0.999
	Class II	12 (60)	18 (82)	0.237
	Class III	6 (30)	4 (18)	0.527

n- number of patients.
* Statistical significant differences between the groups by Mann-Whitney test or Chi-square test ($p < 0.05$); HTA- reported controlled hypertension, OT CVD- other cardiovascular diseases, HTy- Hashimoto thyroideus.

All patients had acrylic dentures (62% complete, 38% partial), and 32% had previous relining procedures performed (Table 2). Nearly 80% of patients, equally distributed among groups, reported they had continuously worn their dentures, especially during the night (Table 2). Almost 90% of the patients in both groups presented with poor oral

Table 2
Risk factors regarding the denture in the patients with diagnosed denture stomatitis at baseline*.

		Test group (n = 20)	Control group (n = 22)	p -value
History of denture relining, n (%)		8 (40)	6 (27)	0.593
Time interval of denture wearing in years, mean ± SD		5 ± 2	4 ± 2	0.364
Frequency of denture wearing; n (%)	Nocturnal denture wearing week (Answered with Yes)	16 (80)	18 (82)	0.564
	Taking off for a couple of hours (Answered with Yes)	7 (35)	5 (23)	
	Never take the denture off (Answered with Yes)	9 (45)	13 (59)	
Oral hygiene denture maintenance; n (%)	Water irrigation	5 (25)	7 (32)	0.366
	Dental brush	6 (30)	7 (32)	
	Dental brush + Tooth paste and Bicarbonate of soda	9 (45)	8 (35)	

n- number of patients.
* Statistical significant differences between the groups by Mann-Whitney test or Chi-square test (p <0.05).

hygiene regimes, in which one-third of patients reported cleaning their dentures only with water or using dental brushes.

Among all patients, *C. albicans* (100%), *C. glabrata* (33%), *C. tropicalis* (31%), and *C. krusei* (31%), were isolated prior to the treatment (Fig. 3). The result of both treatment procedures demonstrated substantial reduction in *Candida* spp. at all follow-up visits (p < 0.05). Despite a statistically significant reduction in *C. albicans* and *C. krusei* that was reported within both groups at all follow-up visits (p < 0.05, Fig. 3), both species were isolated in the control group following topical NYS at 3rd (*C. albicans*: 23%, *C. krusei:* 14%), 15th (*C. albicans*: 23%, *C. krusei:* 9%), and 30th day (*C. albicans*: 14%, *C. krusei:* 9%), and exhibited low growth rates (Fig. 4). PDT showed statistically significant reduction and eradication of *C. krusei* and *C. albicans* compared to NYS at all follow-up intervals (p = 0.0176).

During the treatment, clinical examinations revealed substantial improvement regarding inflammation reduction in the test group compared to the control group after 3rd (Fig. 2c) and 15th day following the therapy (p < 0.05, Fig. 1d, 2d). In the control group, 22% of patients presented with erythema on the 15th day after the treatment and on the 30th day after therapy, no inflammation was detected in either group (Table 3).

In terms of denture prognosis following the treatment and time interval of follow-up visits, only 24% of dentures required relining, while 76% required new complete acrylic dentures.

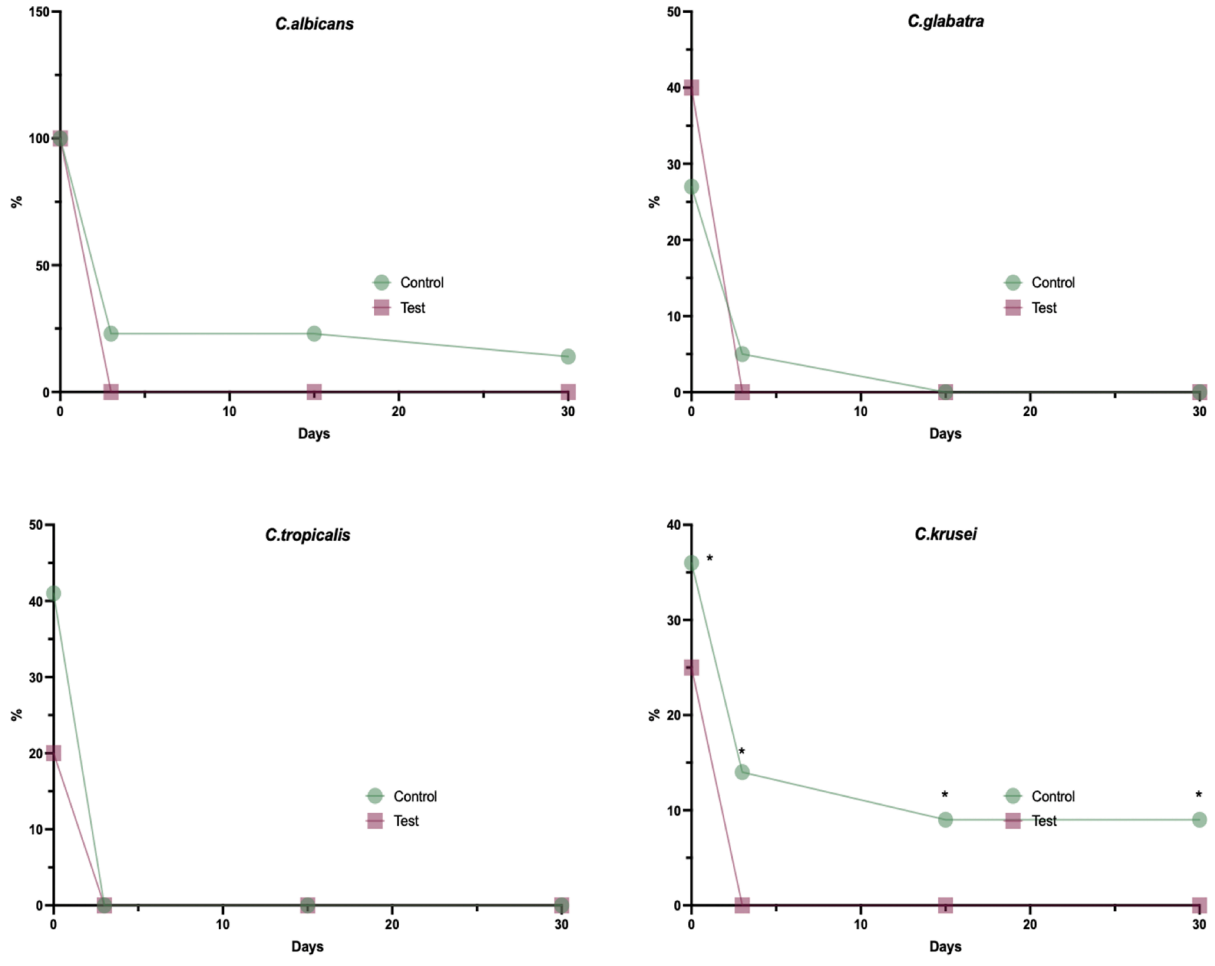

Fig. 3. Distribution of isolated *Candida* spp. between groups before treatment procedure, and on 3rd, 15th, and 30th days following treatment procedures, displayed in percentages.

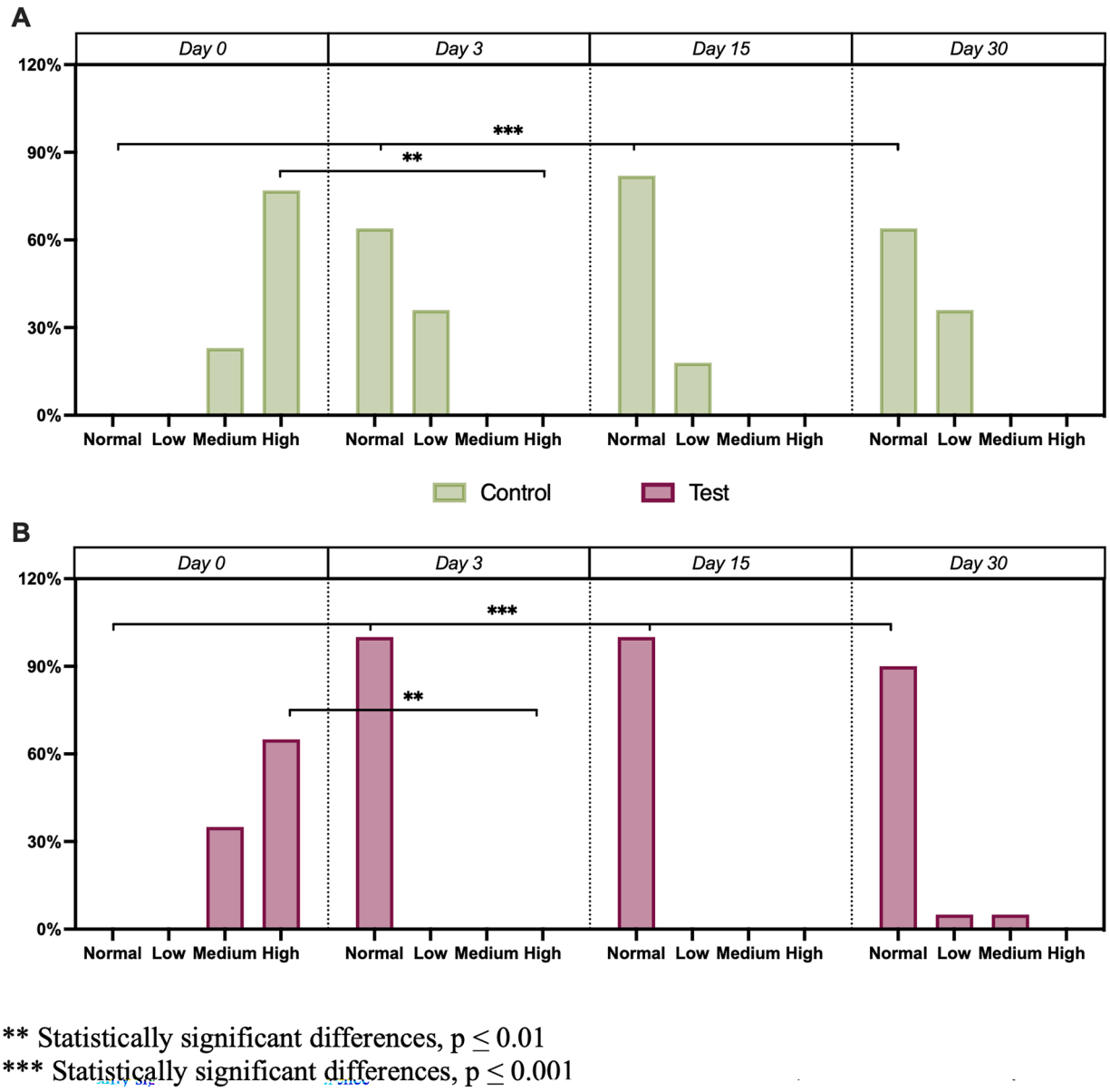

Fig. 4. Growth distribution of *Candida* spp. within the control group (A) and the test group (B) prior to the treatment procedure and at follow-ups, displayed in percentages and marked as normal (no *Candida* spp. detected), low, medium, and abundant growth of *Candida* spp.

Table 3
Clinical outcomes assessment before and following the photodynamic therapy and conventional antifungal treatment.

		Before therapy	*p*-value	3rd day following therapy	*p*-value	15th day following therapy	*p*-value	30th day following therapy	*p*-value
Erythema localized, n (%)	Test group Control group	5 (25) 7 (32)	0.564	2 (10) 12 (68)	0.008 *	0 (0) 5 (22)	0.998	0 (0) 0 (0)	/
Erythema generalized, n (%)	Test group Control group	15 (75) 15 (68)	0.999	0 (0) 3 (14)	0.672	0 (0) 0 (0)	/	0 (0) 0 (0)	/
Hyperplasia, n (%)	Test group Control group	4 (20) 3 (14)	0.705	4 (20) 3 (14)	0.705	2 (10) 3 (14)	0.873	0 (0) 3 (14)	0.257

n- number of patients.
* Statistical significant differences between the groups by Mann-Whitney test or Chi-square test ($p < 0.05$).

4. Discussion

The present prospective clinical study assessed and compared clinical and microbiological outcomes efficacy of a one-session single application of photodynamic therapy (PDT) and topical nystatin oral suspension (NYS) in patients with diagnosed denture stomatitis (DS)

without wearing dentures during treatment and follow-ups. As a result of the study, PDT and NYS significantly demonstrated reduction of the *Candida* spp. and their further growth on the 3rd, 15th, and 30th day following the treatments. Furthermore, when a denture was not worn during the treatment and follow-ups, a one-session single PDT application contributed to a statistically significant reduction in *C. albicans* and *C. krusei* compared to NYS ($p= 0.0176$). These results were comparable to the clinical outcomes improvement and inflammation reduction of oral mucosa on the 3rd and 15th day following PDT.

Denture stomatitis represents a common oral fungal disease with prevalence ranging from 17% to 77% mostly affecting female elderly population [39,40]. However, present results showed no statistically significant difference in the DS occurrence among the female and male population. The microenvironment provided by dentures encourages the growth of *Candida* spp. [41]. Along with pathogenic microbes, several risk factors, including poor-fitting dentures, poor oral hygiene, wearing dentures continuously, especially at the night, and smoking, did significantly influence the DS incidence [42].

Among all *Candida* species, *C. albicans* occurs in high volumes in both dentures and oral mucosa. Other species including *C. krusei, C. tropicalis*, and *C. glabrata* were also isolated showing their involvement in DS [9,10,29]. Similarly, in the present study, high incidences of *C. albicans* (100%) following *C. glabrata* (33%), *C. tropicalis* (31%), and *C. krusei* (31%), were isolated from the palatal mucosa after removal of poor-fitting dentures. On denture surfaces, these pathogens exhibited the production of *Candida* biofilm which contained complex extracellular polysaccharides. This biofilm could infiltrate into mucosal tissue, resulting in an increased resistance to standard antifungal treatments [6,11,42]. These species, in combination with the above-mentioned risk factors, could also contribute to ineffective antifungal treatment outcomes, resulting in *Candida* spp. regrowth and recurrence. Therefore, DS treatment should be based on a multidisciplinary strategy with the aim to reduce treatment time and eradicate *Candida* completely.

In the present study, a one-session single PDT was applied only to palatal mucosa or/and alveolar ridge after removal of poor-fitting dentures, leaving patients without them during all follow-ups. The same strategy was conducted in the control group in which oral suspension of NYS was performed for two weeks as art of the standard DS treatment protocol [43]. In both groups, statistically significant reduction of *Candida* species and their growth was achieved when dentures were not worn during follow-ups. Recent randomized clinical studies demonstrated substantial *Candida* spp. reduction such as *C. albicans* and *C. glabrate* by means of multiple sessions of PDT when both palatal mucosa and denture were simultaneously treated for 15 days [27,37] or four weeks [29,31]. In these studies, the number of isolated *Candida* strains decreased after 15 days following PDT application and conventional antifungal therapy (nystatin oral suspension [27,31] or 2% miconazole gel [29]), supporting the outcomes gained in the present study. However, in most of the cases [27,29,37], *Candida* was not completely eradicated indicating that infections recur within two weeks after antifungal treatment [44]. Conversely, earlier studies showed significant *C. albicans* and *C. glabrata* regrowth which led to disease recurrence after 30 and 45 days following either PDT or topical antifungal therapy (100000IU nystatin oral suspension) [27,37]. Interestingly, in our study, a 14-day NYS treatment showed a trend of reduction in *C. albicans* and *C. krusei*, nevertheless no complete eradication was achieved. Based on these findings, nystatin does not effectively eliminate *Candida*, implying that extended treatment procedures or supplementary methods may be necessary to completely eradicate these pathogens. On the contrary to antifungal NYS therapy, PDT demonstrated successful eradication of *Candida* spp., with substantial reductions in *C. albicans* and *C. krusei*. Additionally, after the 3rd, 15th, and 30th day of PDT treatments, no further growth of *Candida* spp. was detected. Therefore, PDT seems to be more effective at the inactivation of microbes than NYS which agrees with a recent study by Alves et al. [37]. Considering that *C. albicans, C. krusei*, and *C. glabrata* could be suggested to be potential resistance strains to conventional topical antifungal agents resulting in disease recurrence, the gained outcomes imply the possibility of complete *Candida* elimination after performing PDT.

A laser parameter, such as wavelength and dose, as well as photosensitizer concentration and type, could be a possible explanation for the obtained results. To date, there has been no standardized protocol regarding the precise laser parameters used for *Candida* spp. eradication in the DS treatment. The increased energy density (J/cm^2) of the laser leads to decrease *Candida* spp. when different types of photosensitizers were used [45]. According to a recent in vitro study, *Candida* reduction increases with laser energy (dose) [46]. Consequently, 180 J/cm^2 is the recommended energy dose for complete *C. albicans, C. krusei*, and *C. glabrata* elimination, while lower doses (120 J/cm^2 and 60 J/cm^2) were not considered ineffective [46]. It is, therefore, possible that the laser energy of 180 J/cm^2 applied in present study can be explained by the total eradication of *Candida* spp., including those susceptible to resistance such as *C. krusei* and *C. glabrata*. These outcomes were contrary to other studies utilizing 28 J/cm^2, 50 J/cm^2, and 122 J/cm^2 laser energies, respectively [27,29,31,37]. Furthermore, in comparison with LED blue light ($\lambda = 455$ nm), a suitable red laser light with a $\lambda = 600$ to 800 nm applied with a proper optical fiber might penetrate deeper tissue layers [47], subsequently eliminating *Candida* infiltrated into the tissue. This could explain, additionally, no further *Candida* growth 30 days following PDT when 3-D optical fiber of 660 nm of wavelength was employed in the study compared to recent study in which $\lambda = 455$ nm was utilized [27]. Furthermore, the phenothiazine derivative promoted photodynamic inactivation, resulting in a substantial reduction in the viability of *C. albicans, C. glabrata, and C. tropicalis*, [48] which is consistent with presented results. This study performed a new generation of photosensitizer, phenothiazine-chloride derivative, with a concentration of 9 mg/ml methylene blue, representing a higher concentration of photoactive substance than PS was used in previous studies (450 μg/mL [29]). Accordingly, this higher concentration may shorten the exposure time for dyeing *Candida* combined with proper irradiation time and dose; however, further clinical, and experimental studies will be needed to confirm this statement.

In light of the need for a DS's multidisciplinary treatment approach in the *Candida* spp. elimination, considering risk factors such as denture fit, frequency of denture wearing, and oral hygiene regime, the question arises whether dentures should be removed completely and replaced with new ones during and following the treatments [27,49]. According to Budtz-Jorgensen and Bertram [50], poor-fitting denture was associated with trauma, localized inflammatory response to the oral mucosa, and microbial biofilm development. Considering these facts, only patients with poor-fitting dentures were included in this study. Additionally, dentures' rough and porous structure may make it difficult to completely eliminate *Candida* spp. Therefore, constant denture wearing during and after treatments performed may be responsible for incomplete *Candida* elimination and subsequent its regrowth and disease recurrence [27,37]. Accordingly, this was additional reason why patients were asked not to wear their dentures during treatment and follow-ups in the present study. Present outcomes demonstrated that the effective *Candida* spp. elimination and inflammation decrease, especially after PDT, could be attributed to not wearing the denture and replacing it with a new denture (80% of cases) or relining the previous one. As a result of these findings, it might be possible to shorten the time of the therapeutic procedure by means of PDT, allowing patients to obtain new denture in a shorter time span. A long-term randomized clinical study, however, is needed to confirm this statement.

In present study, almost one-third of patients were smokers. Smoking is considered as one of the fundamental local risk factors that influences not only DS onset but also treatment outcomes [51]. Increasing cigarette substance concentrations resulted in biofilm formation, which promoted *C. albicans* growth in vitro [52]. PDT has shown to be an effective treatment for oral diseases including DS, in both non-smokers and smokers [53]. Nevertheless, recent study found that, despite significant

reductions in *Candida* levels between smokers and non-smokers after three months, smoking resulted in *Candida* regrowth [53]. Our research yet found no further growth of *Candida* in the PDT group compared with the control group which was contrary to Senna et al. study [29] in which antifungal conventional therapy was more effective compared to PDT. The possible explanation of these outcomes might be by the unequal distribution of smokers between groups (only three patients in test group) as well as the shorter follow-up period (30 days) in comparison with the long-term follow-up (up to three months) of the recent study [29], which might be one of the study's limitations, respectively.

Another study limitation could be the inability to compare colony counts by species, despite both conventional therapy and PDT demonstrating a significant reduction in *Candida* spp. The documented *Candida* resistance species and the possibility of disease recurrence make the detection of colonies before and after treatment important for the resistant species, including *C. krusei*. In comparison to conventional antifungal treatment, it might be interesting to know how much *Candida* colony reduction could be expected in the patient with diagnosed DS who does not wear dentures following PDT. A long-term study is necessary to address this issue.

5. Conclusion

Considering study limitations, PDT can effectively eliminate *Candida* spp. in patients with dentures stomatitis after removing inadequate dentures and replacing or relining them, improving clinical parameters, and enhancing patients' quality of life in the short span. Accordingly, PDT might be considered an effective therapeutic approach in the denture stomatitis treatment.

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CRediT authorship contribution statement

Dragana Rakasevic: Conceptualization, Methodology, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Jelena Marinkovic:** Investigation, Data curation, Conceptualization. **Bojan Rakonjac:** Formal analysis, Data curation. **Marion Arce:** Software, Formal analysis. **Ena Joksimovic:** Writing – review & editing, Investigation, Data curation. **Jovana Markovic:** Writing – review & editing, Investigation, Data curation. **Milan Kulic:** Writing – review & editing. **Milos Hadzi-Mihailovic:** Supervision, Investigation, Conceptualization. **Aleksa Markovic:** Supervision, Investigation, Conceptualization.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pdpdt.2023.103913.

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