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ORIGINAL ARTICLE

Can 0.5% Sodium Hypochlorite Treat *Candida*-Associated Denture Stomatitis?

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ABSTRACT

Objective: To evaluate a 0.5% sodium hypochlorite (SH) protocol in reducing *Candida spp.* levels in complete dentures (CD) and palate and denture stomatitis (DS) remission. **Material and Methods:** Twelve CD wearers diagnosed with *Candida*-associated denture stomatitis (CADS) had their initial situation (*Candida* spp. levels and DS score) recorded (baseline). Then, participants were instructed to soak dentures once a day (10 minutes) in 0.5% SH. *Candida* spp. levels and DS scores were reassessed after 15, 30, and 60 days of SH denture cleanness. Biofilms from the denture base and palate were seeded in CHROMagar *Candida*. After incubation, colony-forming units were calculated. The palate was photographed at each time point, and DS was assessed according to Newton's classification. Data of *Candida* spp. levels were analyzed by 2-way repeated measures ANOVA followed by the Holm-Sidak test, and DS scores data were accessed by Friedman's 2-way ANOVA by ranks (α=0.05). **Results:** 0.5% SH significantly reduced *Candida* spp. levels after treatment compared to baseline ($p<0.001$) for both sites. Although at baseline, *Candida* spp. counts were higher on the denture base ($p<0.001$), no significant differences were observed between the collected areas within the other time points ($p<0.05$). Also, 0.5% SH effectively reduced clinical signs of DS after treatment ($p<0.05$). **Conclusion:** The protocol tested effectively decreased *Candida* spp. levels on the denture base and palatal mucosa and effectively reduced the signs of DS.

Keywords: Sodium Hypochlorite; *Candida*; Stomatitis, Denture.

Introduction

Denture stomatitis (DS) is an inflammatory disorder observed in the tissue underlying a complete denture, mainly in the palatal mucosa [1,2]. Generally asymptomatic, it affects one in every three denture wearers [3]. *Candida* spp. biofilm formed on the denture base is the main etiological factor of DS [4]. Commonly used denture resins are a favorable substrate for *Candida* spp. proliferation and protect them from saliva and chemical agents [5,6].

Clinicians first choose antifungals to treat *Candida*-associated denture stomatitis (CADS); however, high recurrence rates and *Candida* species recolonization have been reported after therapy cessation [1]. Against this, repeated use of antifungals is required, exposing patients to adverse effects and resistance to medication [7]. Considering the limitations of antifungals in controlling mucosa infection, effective removal of the main etiological factor from the denture base seems to be the best alternative to treat CADS [8].

Soaking dentures in chemical products seems to be the best hygiene method, especially for patients with limited motor capacity [9,10]. Easy to handle, nontoxic [11], low cost, and effective in removing organic and inorganic deposits, sodium hypochlorite (SH) is the most effective chemical product to eliminate *Candida* spp. biofilm [5]. As this cleanser affects denture materials in high concentrations [12,13], studies are testing it at lower concentrations for shorter exposure times [14,15].

Studies have shown that 0.1-0.5% SH is still efficient in decreasing *Candida* spp. levels without damaging denture surfaces [5,14,16,17]. On the other hand, the protocols testing this chemical product at very low concentrations (0.1-0.25%) could not promote a complete remission of DS signs [16,18-21]. Thus, this prospective clinical study aimed to evaluate a hygiene protocol with SH at 0.5%, using a shorter time of immersion (10 minutes), in reducing *Candida* spp. levels in maxillary complete dentures and palate and DS remission. The null hypotheses were that (I) the periods of evaluation and collected areas would not interfere with *Candida* spp. levels, and (II) the evaluation periods would not interfere with DS clinical signs.

Material and Methods

Study Design, Ethical Clearance and Volunteer's Selection

This prospective clinical study had a double-blinded design (participants and researchers regarding DS classification and CFU counts) and was approved by the local Research and Ethics Committee (no. 1.548.729). The study sample consisted of maxillary complete dentures wearers who attended the Removable Dental Prothesis Clinic in the School of Dentistry of the Federal University of Maranhão (São Luís, MA, Brazil) and have been diagnosed with CADS. The inclusion criteria for participation were good general health, not taking antimicrobial agents (antibiotics or antifungals), or using mouthwash solutions three months before the study; the dentures could not be relined, repaired, or fractured. Also, the subjects who used any chemical product to clean their dentures were not included in the study.

The sample size estimation was based on *Candida* spp. levels obtained from a pilot study using tests with a power of 80% and α=0.05 (Power Analysis—Related Sample Means, SPSS Statistics software version 26, IBM Corp, Chicago, IL, USA).

Intervention (Treatment)

After all participants had signed written informed consent forms, the patient's initial situation (baseline) for the two outcome variables was assessed: *Candida* spp. levels and DS classification. Then, participants were instructed (verbal and written) to soak their dentures for 10 minutes, once a day (at night, after the last meal), in

200 mL of 0.5% sodium hypochlorite solution (Alquimia Pharmacy, São Luís, MA, Brazil). They were given no instructions regarding denture brushing as all participants related cleaning their prostheses by brushing with soap or dentifrice. They were oriented not to discontinue their mechanical method, and the researchers did not interfere with the nocturnal denture wear habit. *Candida* spp. levels and DS classification were reassessed three times after 15, 30, and 60 days of treatment.

DS Evaluation

To evaluate denture stomatitis levels, a researcher photographed the participants' palate using a digital camera (Sony Alpha DSLR A200; Sony Corporation, Tokyo, Japan) and macro lens (Sony 2.8/100; Sony Corporation, Tokyo, Japan). Each photograph (baseline and time points) was analyzed and scored separately by two independent researchers according to the Newton classification [22], as follows: 0: rosy mucus, normal vascularization, and matte appearance; 1: reddish mucus, solitary focus of hyperemia, and matte appearance; 2: reddish mucus, multiple hyperemic focus, and shiny; and 3: clearly red or red to blue and shiny. The classifications of both researchers were compared, and any differences were discussed until agreement was reached [16,18,21].

Candida spp. Levels

Candida spp. levels were quantified by the colony forming unit (CFU) numbers on dentures and palate. Biofilm on the internal denture surface and hard palate was mechanically collected by swabbing each area separately for 1 minute each; then, the biofilm was directly spread onto a petri dish containing a selective growth medium for *Candida* spp. (BBL CHROMagar *Candida*; Becton Dickinson, Franklin Lakes, NJ, USA) [23,24]. The inoculated samples were incubated in an oven at 37°C for 48 hours for fungal growth, and then, the number of colony-forming units (CFUs) was quantified using the stereomicroscope.

Statistical Analysis

The data were analyzed using SPSS Statistics software version 26 (IBM Corp, Chicago, IL, USA), with a significance level fixed at 5%. Data of *Candida* spp. levels and DS scores were not normally distributed (Kolmogorov-Smirnov test). So, *Candida* spp. levels values were logarithmically transformed, and factors interfering in the response of this variable (collected areas and periods of evaluation) were analyzed using two-way repeated measures ANOVA. Post hoc comparisons were performed using the Holm-Sidak test. Data on the DS score were accessed by Friedman's 2-way ANOVA by ranks to identify differences among evaluation periods.

Results

Seventeen patients were examined – three did not meet all inclusion criteria, and two volunteers were excluded during the experiment because they did not perform the given hygiene protocol correctly. Thus, the final sample of this study consisted of 12 participants (1 man and 11 women) with a mean age of 68 years (range 54 to 84 years). The number of subjects per the preliminary sample calculation indicated that 11 volunteers would be sufficient to detect significant differences.

Two-way repeated measures ANOVA found a statistically significant reduction of *Candida* spp. levels after use of 0.5% SH ($p<0.05$). Also, considering this response variable, statistically significant interactions were found between areas and periods of evaluation ($p=0.003$). Comparisons among evaluation periods within each collected area found that the baseline presented a statistically higher number of *Candida* spp. CFU than the other time points ($p<0.001$). On the other hand, *Candida* species levels recorded after 15, 30, and 60 days of SH

treatment did not differ significantly from each other (p>0.05). At baseline, *Candida* spp. counts were significantly higher on the denture base than on the palate (p<0.001); however, no significant differences were observed between these collection areas in the evaluation periods after SH treatment (p>0.05) (Table 1).

Table 1. *Candida* spp. levels (CFU) on collected areas at baseline and after using 0.5% SH.

Area	Periods of Evaluation			
	Baseline Mean ± SD	15 Days Mean ± SD	30 Days Mean ± SD	60 Days Mean ± SD
Palate	247.8 ± 390.8^{Aa}	1.1 ± 2.3^{Ba}	2.8 ± 5.9^{Ba}	0.8 ± 1.4^{Ba}
Denture Base	1771.9 ± 464.2^{Ab}	158.8 ± 359.9^{Ba}	150.3 ± 475.4^{Ba}	18.8 ± 40.7^{Ba}

Different uppercase letters represent statistically significant differences among evaluation periods, and different lowercase letters represent differences between collected areas (Two-way repeated measures ANOVA; Holm-Sidak; p<.05).

Considering the response variable DS score, Friedman 2-way ANOVA by ranks showed significant differences among evaluation periods (p<0.05). Table 2 shows a reduction in DS scores after denture cleanness with SH since all other periods significantly differed from baseline (p<0.05) (Figure 1). However, no statistical differences were observed among evaluation periods after treatment (p>0.05).

Table 2. Percentage of DS scores at baseline and after using 0.5% SH.

DS Score	Periods of Evaluation			
	Baseline*	15 Days#	30 Days#	60 Days#
0	-	58.3%	91.7%	91.7%
1	25.0%	41.7%	8.3%	8.3%
2	58.3%	-	-	-
3	16.7%	-	-	-

Different symbols represent statistical differences among evaluation periods (Friedman 2-way ANOVA by ranks; p<.05).

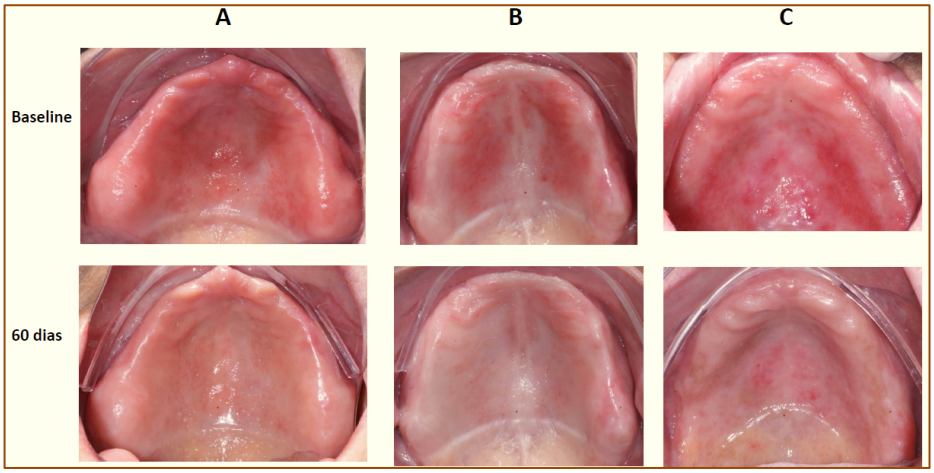

Figure 1. Palatal mucosa at baseline and after 60 days of 0.5% SH use.

Discussion

The null hypotheses were rejected because using 0.5% SH effectively reduced clinical signs of DS and decreased *Candida* spp. levels on denture base and palatal mucosa after treatment compared to baseline.

Although SH is known as an excellent antimicrobial solution, using this product as a denture cleanser has not been recommended since some authors found that SH could damage denture materials [12,13,15]. However, other studies have demonstrated that soaking dentures in SH solution with reduced concentrations and for shorter periods does not affect the denture surface. In contrast, the effectiveness against denture biofilm is maintained [5,14,17]. Besides the concentration of SH solution and immersion period, the frequency of denture

exposure to SH seems to be an important factor in its antimicrobial effect. Valentini-Mioso et al. [10] reported no reduction in *Candida* spp. levels after treatment with 0.5% SH. This can be justified because, unlike other studies, in that study, dentures were not immersed daily in SH but only once a week for two weeks. As SH doesn't have a residual antimicrobial effect of eliminating the recolonization of acrylic resin [11], dentures must be soaked daily in low concentrations of this solution. Thus, in the present study, the protocol tested was the daily immersion of 0.5% SH for 10 minutes.

Regarding *Candida* species, the present cleansing routine significantly reduced the levels of these fungi, following other clinical studies testing 0.1-0.25% SH daily use [16,18-21]. Although SH concentration is lower in these studies, denture soaking is longer (20 minutes). A shorter immersion time, as adopted in the present study (10 minutes), is important when establishing a daily cleansing protocol since reducing the time spent soaking dentures by half may contribute to patient adhesion to treatment.

Even though *Candida* spp. levels before treatment (baseline) were higher on the denture base than on the palate, regardless of DS classification [22], no significant differences in these fungi levels were observed between the collected areas within the evaluation periods after treatment. These findings are important since the denture hygiene protocol employed was able to eliminate *Candida* spp. from both sites. Some studies are concerned with removing palate biofilm to reduce *Candida* spp. levels and treat CADS [8,21]; on the other hand, the present study focused on denture cleanness because (a) effective palatal biofilm removal requires a degree of manual dexterity often lacking among geriatric patients and those with limited motor capacity; (b) soaking dentures in cleansing solution is easy to handle, and (c) biofilm formed on denture base is the main etiological factor for the development of CADS since this surface provides a suitable micro-environment for *Candida* species proliferation [5,6].

Control of *Candida* spp. levels is essential because it has been directly related to DS [21]. Even though antifungals are the first choice to treat CADS, they must be used for a limited period because of adverse effects, with high CADS recurrence rates after cessation of therapy [1]. Thus, an alternative is treatments focused on the daily elimination of denture biofilm, DS's main etiological factor [7], and SH is considered the gold standard denture cleanser solution in eliminating *Candida* spp. Biofilm [5,20] studies are testing protocols using this chemical product to reduce DS signs. These studies [16,18-21] have observed a significant reduction in DS clinical signs after 10-14 days of treatment but not a complete reduction (score 0) [22]. At a similar period of treatment (15 days), the present showed better results, with a significant number of volunteers without DS signs (58.3%) and no volunteer presenting a DS score of two or three. This may have occurred because the previous studies employed a 0.1-0.25% SH concentration, while the present study tested a higher concentration (0.5%).

Most parts of the studies tested SH against DS for 10-14 days [16,19-21], while the present research treated and accompanied the patients for 60 days. It was important because, for some volunteers, a more extended period of SH use was necessary to eliminate signs of CADS. Even though after 30 days, almost all patients did not present DS signs (91.7%), the observation made with 60 days of treatment was essential to evaluate if the palate health would have been maintained, and it was. Only one volunteer (8.3%) did not completely reduce palatal inflammation, even after 60 days of SH use (Figure 1-B). On the other hand, this patient had a reduction in the degree of DS after 15 days of use (score 2 at baseline to score 1) and a significant decrease in *Candida* species levels, which, after 60 days, was not detected by the microbiological exam. Since for this patient, *Candida* spp. was not playing a role in DS; another etiological factor should be maintaining a solitary focus of hyperemia, like a possible trauma of the unstable denture to the palatal mucosa [8].

In this study, researchers did not interfere with volunteers' habits, such as overnight dentures and mechanical cleanness. Although nocturnal removal of the dental prosthesis has been recommended in treating DS [2,3,16,18-21], it can be embarrassing for the patient [10]. On the other hand, this study has shown that patients can sleep with the prosthesis as long as it is effectively clean. Since mechanical cleansing was not effective for the volunteers, SH was responsible for *Candida* species biofilm control.

Although *Candida* spp. are considered the main pathogen of DS due to their ability to form biofilms on denture surfaces, bacteria are also associated with the disease [4,10]. A limitation of the present study was not being able to assess the behavior of these microorganisms in relation to treatment with SH since the microbiological analysis employed [23,24] does not enable quantitation of other microbes in addition to *Candida* spp.

Conclusion

Denture daily immersion in 0.5% sodium hypochlorite for 10 minutes effectively decreased *Candida* spp. levels on the denture base and palatal mucosa and reduced the signs of denture stomatitis during the whole evaluation period.

Authors' Contributions

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All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support this study's findings can be made available upon request to the corresponding author.

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