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ORIGINAL RESEARCH

Effectiveness of a Chlorhexidine Digluconate 0.12% and Cetylpyridinium Chloride 0.05% Solution in eliminating *Candida albicans* Colonizing Dentures: A Randomized Clinical *in vivo* Study

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ABSTRACT

Background: Effective denture hygiene is important for patients suffering from denture stomatitis (DS). This study aimed to evaluate the efficacy of a solution containing 0.12% chlorhexidine (CHX) digluconate and 0.05% cetylpyridinium chloride (CPC) in eliminating *Candida albicans* colonizing dentures.

Materials and methods: Forty denture wearers (11 men, 29 women; age range 40 to 80 years) with clinical evidence of DS were randomly divided into two groups, one test and one control. The dentures of the test group were treated by immersion in a solution of 0.12% CHX and 0.05% CPC while those of the control group were immersed in distilled water. Swabs were collected from the fitting surfaces of the upper dentures prior and post cleaner use and examined mycologically.

Results: Reduction in the number of colony-forming units (CFU) of *Candida albicans* after immersion of the dentures in a solution of 0.12% CHX and 0.05% CPC was significantly greater than that of the control group.

Conclusion: A solution of 0.12% CHX and 0.05% CPC tested as a product of disinfection of the acrylic dentures showed significant results after immersion of 8 night hours for 4 days.

Keywords: *Candida albicans*, Cetylpyridinium chloride, Chlorhexidine digluconate, Denture stomatitis.

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INTRODUCTION

Denture stomatitis (DS) (chronic erythematous candidiasis) is a chronic inflammatory lesion of the denture bearing mucosa found in approximately 50% of patients wearing complete or partial dentures. It is more common in women than men and the palatal mucosa is the most affected.[1] It has a multifactor etiology, such as trauma caused by the denture itself, continuous denture wearing, oral and denture poor hygiene, dietary and systemic factors.[2,3]

Candida albicans among other species of fungus colonizing dentures are important agents for the installation, maintenance and exacerbation of this disease.[4] They are found in biofilm highly adherent to the base material of dentures. The presence of microfissures and cracks within the material and an increased surface roughness will also increase their colonization.[5]

Normally the mucosal surface provides an effective barrier to infection; however, an ill-fitting denture may cause frictional irritation of the palatal mucosa, and thus facilitates invasion of *Candida* into the superficial layers of the epithelium.[6]

In this respect, daily cleansing of dentures is an important factor in the elimination of the biofilm formation.[7]

The efficacy of several denture-cleansing techniques has been clinically evaluated and literature reported

substantial data on mechanical brushing, microwave sterilization and the use of chemical cleansing like soap, effervescent tablets and mouthwashes.[8,9]

The aim of this study was to investigate the efficacy of a mouthwash combining two antiseptics, chlorhexidine digluconate 0.12% (CHX) and cetylpyridinium chloride 0.05% (CPC), in reducing or eliminating *Candida* biofilm of denture associated with DS compared to distilled water as control solution.

Chlorhexidine is an antiseptic active against various bacteria, viruses, and fungi including *Candida albicans*.[10] Its clinical and microbiological efficacy in treating DS has been proven.[8,11-13] However, the brown coloration of the acrylic denture soaked in CHX alone can be a major obstacle in using it as a daily decontamination product.[12]

Cetylpyridinium chloride is an antiplaque and toxin-neutralizing for maximum antimicroorganism protection.[14,15] Its high advantage is the low coloration and staining degree compared to CHX.[15]

The reinforcement of CHX with CPC helps neutralize the toxins released by microorganisms in the mouth. Indeed, when microorganisms are killed and disaggregate, they release proinflammatory toxins called lipopolysaccharides, and their elimination is thus, an essential component of a complete antibacterial therapy.

MATERIALS AND METHODS

This randomized controlled trial was conducted in accordance with the Helsinki agreement for research on humans, and the study design was approved through the Institutional Review Board and Independent Ethics Committee of the School of Dentistry, Lebanese University, Beirut, Lebanon. Written informed consent was obtained from all participants in the study prior to treatment.

Complete maxillary edentulous denture-wearing patients attending the Department of Oral Pathology and Diagnosis at the Lebanese University, during a period of 1 year, were examined for clinical evidence of Newton's type II denture stomatitis. Newton´s type II is a diffuse erythema involving part or all of the mucosa, which is covered by the denture.[16]

We included in the study, patients who were: (1) confirmed having *Candida albicans* in their dentures, (2) aged between 40 and 80 years, (3) healthy, (4) not taking any medication that might affect the oral bacterial flora, and (5) wearing the maxillary full prosthesis for more than 1 year.

Patients with systemic diseases, such as diabetes, nutritional deficiencies and those wearing their dentures for less than 1 year were excluded.

Forty patients met the inclusion criteria. They were randomly assigned to one of 2 groups (test and control), of 20 patients each and were asked to avoid cleaning their dentures during the experimental procedure to standardize the study.

A quantitative microbiological measurement was performed the first day (D1) from the infected oral mucosa and the fitting side of the dentures.

Patients in the test group had their dentures soaked in a solution of CHX 0.12% and CPC 0.05% (Paroex°; Sunstar: Butler, USA) during the night for 8 hours (from 10 pm to 6 am) for four consecutive nights, the ones of the control group in distilled water following the same protocol.

A second swab collection destined for a new *Candida albicans* colony count was taken on the day 4 (D4).

One investigator carried out microbiological procedures. The Becton Dickinson (New Jersey, USA) Microbiology System, BBL CultureSwab was used. These systems are sterile devices for collecting and transporting microbiological specimens (Amies, Stuart and agar gel).

Swabs were cultured in Sabouraud's dextrose agar (40 g/l dextrose, 10 g/l peptone and 20 g/l agar) and containing chloramphenicol 0.5 g/l and actidione 0.5 g/l. *Candida* count was carried out after 48 hours incubation at 37°C in aerobic conditions. *Candida albicans* was differentiated from the other species by their production of filaments in 0.5 ml of animal serum.

The primary outcome measure was the relative reduction in *Candida albicans* colony count expressed in CFU/ml (colony-forming unit) collected from the denture surface at Day 1 (D1) and after the 4 nights of immersion at day 4 (D4). The relative reduction = (a - b)/a, 'a' being the number of colonies before immersion and 'b' the number of colonies after.

The Kolmogorov-Smirnov test was used to assess the normality of distribution and the data was found to follow a non-normal distribution. Accordingly, the nonparametric Kruskal-Wallis test was applied to test the working hypothesis of difference in the relative reduction of *Candida albicans* between the two groups and a comparison procedure (Mann-Whitney) was performed to analyze the data.

A confidence level of 0.05 was considered statistically significant. Data were analyzed using the statistical package for social sciences (SPSS) (IBM, USA), version 21.0.

RESULTS

Compared at baseline, no significant statistical difference was noted in the mean age ($p = 0.124$), *Candida albicans* count on the palate ($p = 0.516$) and on the dentures ($p = 0.484$). Characteristics of the two patients groups were summarized in Table 1.

Table 1: Descriptive statistics of the two study groups

Group		*Age*	*Palate-D1*	*Denture-D1*	*Denture-D4*	*Relative reduction*
(C+C)* (n = 20)	Mean (SD)	66.65 (7.68)	2280.4 (4036.65)	2911 (3753.48)	8 (18.807)	0.991 (0.002)
	Med (min, max)	68.5 (48, 76)	260 (20, 13000)	1300 (40, 12000)	0 (0, 80)	1 (0.993, 1)
DW** (n = 20)	Mean (SD)	66.4 (11.49)	1954 (4320.5)	252717.1 (442673.38)	253568.25 (442172.21)	–1.371 (4.271)
	Med (min, max)	68 (46, 81)	290 (20, 18000)	2750 (40, 100000)	7350 (40, 100000)	0 (–18.37, 0.388)

*CHX and CPC; **Distilled water

When comparing the relative reduction of *Candida albicans* on the dentures (CFU/ml) between the two groups, a significant statistical difference was noted (χ^2 = 48.678, p < 0.0001). The CHX and CPC solution showed the greatest relative reduction with a sum of ranks of 985, followed by distilled water (sum of ranks = 224) (Table 2).

Additionally, the sum of ranks were compared by the Wilcoxon-Mann-Whitney test and, the results showed that CHX and CPC solution was significantly more effective than distilled water (p < 0.0001) (Table 3).

DISCUSSION

Amongst the most important fungal pathogens are yeast species belonging to the genus *Candida*, which encompasses highly virulent pathogens and responsible for the majority of fungal infections. DS is a common infection associated with *Candida albicans*. A study of Budtz-Jørgersen et al (1996) detected DS in 72% of denture wearers in an elderly population living in a geriatric institution.[17] It has been widely accepted that proper routine cleansing of dentures is required to prevent DS and maintain healthy supporting tissues.[18] Kulak-Ozkan et al (2002) evaluated 70 complete denture wearers clinically and mycologically. They concluded that there exists a statistically significant relationship between DS, presence of yeasts and denture hygiene.[19] It is well accepted that chemical disinfectants are more easy to use and effective than mechanical cleaning.[20]

Table 2: Comparison of the relative reduction in *Candida albicans* between the two groups

Group	*Sum of ranks*	*Chi-square*	*Degree of freedom*	*p-value*
CHX and CPC	985	48.678	2	< 0.0001*
Distilled water	224			

*Significant, p < 0.05

Table 3: Comparison between the two groups considering *Candida albicans* colony counts before and after treatment using Wilcoxon rank sum test

Immersion	*Sum of ranks*	*Mean rank*	*p-value*
CHX and CPC	610	29.5	< 0.0001*
Distilled water	210	10	

*Significant, p < 0.05

This study investigated the effectiveness of the association of two antiseptics solutions, CHX 0.12% and CPC 0.05%, in eliminating *Candida albicans* on dentures compared to a control solution, distilled water.

CHX 0.2% has been successfully used as a mouth rinse in the treatment of *Candida*-associated DS,[21] and has been widely considered as the antiseptic of choice for decontaminating the dentures infected by *Candida albicans*.

For Pavarina et al,[11] 10 minutes of complete dentures immersion with a solution of CHX 4% was totally efficient in the elimination of *Candida albicans*. However, CHX 4% solution affected negatively the hardness and roughness of acrylic resins.[22]

After testing at lower concentrations and periods (2% and 90 seconds respectively), Pellizzaro et al[18] found that the antimicrobial efficacy was sufficient to completely inactivate *Candida albicans* on an acrylic resin. The latter corresponds mainly to the chemical reaction of the solution against fungal cell. MacNeill et al[23] observed that post-contact with CHX, *Candida albicans* cells revealed severe cytoplasmic degeneration, fragmentation and desquamation of the cell wall causing in cell death.

Regardless the efficiency, the use of CHX is deemed limited due to its brown stains coloration on the acrylic resin.[12,13]

As for CPC, its antifungal efficacy has been proven *in vitro* and *in vivo* by the reduction of the adhesion of *Candida albicans* on the epithelial cells of human mucosa by 80%, showing efficacy similar to that of 0.2% CHX, which achieved a 77% reduction.

Other advantages of CPC are the lower risk of developing microbial resistance and of causing drug interactions and the low coloration and staining degree compared to the CHX.[15]

In our study, over the 20 patients of the first group, dentures soaked in 0.12% CHX and 0.05% CPC, 15 showed a total disappearance of *Candida albicans* (0 CFU/ml) after four nights of immersion (D4), 4 registered a decline in the number of colonies to 20 CFU/ml (result within normal limits) and only one kept an elevated count of 80 CFU/ml. It is worth noting that this last patient had an initial count

of 12000 CFU/ml. Therefore, the brown coloration was noted on all first group patients' prostheses.

As for the control group, as expected, the results showed absolute ineffectiveness of the distilled water.

CONCLUSION

Significant antifungal effect of chlorhexidine digluconate 0.12% and cetylpyridinium chloride 0.05% solution is noted when used as a disinfection product for infected dentures by *Candida albicans* after soaking them for 8 hours during 4 consecutive nights.

However, the resin staining problem deserves subsequently in depth research, under various operating protocols.

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