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Original Research

Effectiveness of Hexetidine 0.1% in Eliminating *Candida albicans* Colonizing Dentures: A Randomized Clinical *In Vivo* Study

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Abstract:

Background: Effective cleaning of dentures is important to maintain a good oral hygiene for patients suffering from denture stomatitis (DS). This study aimed to evaluate the efficacy of hexetidine 0.1% in eliminating *C. albicans* colonizing dentures.

Materials and Methods: A total of 40 denture wearers (18 men, 22 women; age range 40-80 years) with clinical evidence of DS were randomly divided into 2 groups, 1 test, and 1 control. The dentures of the test group were treated by immersion in hexetidine 0.1% while those of the control group were immersed in distilled water. Swab samples from the palatal surfaces of the upper dentures were collected before and after of cleaner use and examined mycologically.

Results: Reduction in the number of colony-forming units (CFU) of *C. albicans* after immersion of the dentures with hexetidine 0.1% was evaluated compared to those of the control group.

Conclusion: Hexetidine 0.1% solution tested for the first time as a product of disinfection of the acrylic dentures showed average results after immersion of 8 night hours for 4 days.

Key Words: *Candida albicans*, denture stomatitis, hexetidine

Introduction

Denture stomatitis (DS) is defined as an inflammatory process of the oral mucosa underlying a removable, partial or total, dental prosthesis.[1] Etiological factors in DS include the trauma caused by an ill-fitting denture, lack of oral and prosthesis hygiene and a favorable environment for the proliferation of microorganisms.[2] Although bacterial infection, mechanical irritation, and allergic reaction have been proposed as possible causes of DS, infection caused by the overgrowth of fungal species in the genus *Candida* mainly *C. albicans* is often implicated.[1] For Gendreau and Loewy, 15-70% of denture wearers have DS and oral hygiene related risk factors of this condition are significantly associated with morbidly increased colonization of *C. albicans*.[3]

Denture hygiene is an important factor in the prevention and treatment of DS. For that, many modalities of oral care and denture-cleansing techniques have been suggested such as mechanical brushing, microwave sterilization and the use of chemical cleansing like soap, effervescent tablets, and mouthwashes.[4,5]

Among the mouthwashes, many have been suggested as soaking solutions such as, cetylpyridinium chloride, chlorhexidine digluconate,[4-9] and hexetidine..

Jones *et al.* (1997) described the positive effect of hexetidine in decreasing the adherence of the *C. albicans* in the epithelial cells of the oral mucosa.[10] This adherence is a key factor in the filamentation and therefore in the transition to the virulence of *Candidas* generally saprophytes. In addition, hexetidine 0.1% is available at affordable prices in the local market. This will enable the patients, in case this solution was deemed effective, to use it as part of a daily prosthetic hygiene.

However, studies of the hexetidine effect on *C. albicans* are rare and to our knowledge, its *in vivo* effect on *C. albicans* of the acrylic dentures has not been investigated yet.

The aim of this study was to test the efficacy of hexetidine mouthwash in reducing or eliminating *C. albicans* associated with DS compared to distilled water as a control solution.

Material 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 DS. Newton's type II is a diffuse erythema involving part or all of the mucosa, which is covered by the denture.[11]

We included in the study, patients who were: (1) Confirmed having *C. 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 1st 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 hexetidine 0.1% during the night for 8 h (from 10 pm to 6 am) for 4 consecutive nights, the ones of the control group in distilled water following the same protocol.

A second swab collection destined for a new *C. 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 Culture Swab 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 0.5g/l. *Candida* count was carried out after 48 h incubation at 37°C in aerobic conditions. *C. 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 *C. albicans* colony count expressed in colony-forming unit (CFU)/ml 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 were found to follow a non-normal distribution. Accordingly, the non-parametric Kruskall–Wallis test was applied to test the working hypothesis of difference in the relative reduction of *C. 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 (IBM, USA), Version 21.0.

Results

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

When comparing the relative reduction of *C. albicans* on the dentures (CFU/ml) between the two groups, a significant statistical difference was noted ($\chi^2 = 48.678$, $P < 0.0001$). The hexetidine showed the greatest relative reduction with a sum of ranks of 621, followed by distilled water (sum of ranks = 224) (Table 2).

In addition, the sum of ranks were compared by the Wilcoxon-Mann–Whitney test and the results showed that hexetidine was significantly more effective than distilled water ($P < 0.0001$) (Table 3).

Discussion

Fungi are eukaryotic micro-organisms. The most relevant of these belong to the genus *Candida*.[12] Many types of fungal infections occur in the mouth although the most common is the candidiasis caused by *C. albicans*[13] colonizing oral cavity in 40-60% of healthy persons.[14] Dentures predispose to candidiasis in as many as 65% of people wearing dentures which produces a microenvironment conducive to the growth of *Candida* with low oxygen, low pH, and an anaerobic environment. This may be due to enhanced adherence of *Candida* to acrylic, reduced saliva flow under the surfaces of the dentures or poor oral hygiene.[15]

A study of Budtz-Jørgensen *et al*. (1996) detected DS in 72% of denture wearers in an elderly population living in a geriatric institution.[16] It has been widely accepted that proper routine cleansing of dentures is required to prevent DS and maintain healthy supporting tissues.[17] 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 cleanliness.[18] It is well accepted that chemical disinfectants have some advantages over mechanical cleaning such as effective disinfection and ease of use.[19]

This study investigated the effectiveness of hexetidine 0.1% mouthwash, in eliminating *C. albicans* on dentures compared to a control solution, distilled water.

Table 1: Descriptive statistics of the two study groups.

Group	Age	Palate-D1	Denture-D1	Denture-D4	Relative reduction
H (*n*=20)					
Mean (SD)	70.45 (12.38)	905 (2663.967)	52860.5 (222960.16)	6576.4 (26712.55)	0.751 (0.289)
Med (Minimum, Maximum)	75.5 (41, 81)	170 (20, 12000)	2000 (40, 1000000)	185 (0, 120000)	0.847 (−0.2, 1)
W (*n*=20)					
Mean (SD)	66.4 (11.49)	1954 (4320.5)	252717.1 (442673.38)	253568.25 (442172.21)	−1.371 (4.271)
Med (Minimum, Maximum)	68 (46, 81)	290 (20, 18000)	2750 (40, 100000)	7350 (40, 100000)	0 (−18.37, 0.388)

SD: Standard deviation

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

Group	Sum of ranks	Chi-square	Degrees of freedom	*P* value
Hexetidine	621	48.678	2	<0.0001*
Distilled water	224			

*Significant, *P*<0.05

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

Immersion	Sum of ranks	Mean rank	*P* value
Hexetidine	596	28.5	<0.0001*
Distilled water	224	11.05	

*Significant, *P*<0.05

For Sixou and Hamel,[20] the effect of hexetidine on *C. albicans* is variable. Jones *et al.*[10] described the positive effect of hexetidine in decreasing the adherence of *C. albicans* in the epithelial cells of the oral mucosa. A study conducted *in vitro* by Luc *et al.*[4] shows that hexetidine 0.1% is slightly active on the *C. albicans* after contact duration of 5 min.

Our choice was, despite the above, fixed on hexetidine for the following reasons:

1. The studies of the action *in vivo* of hexetidine on *C. albicans* are rare. Moreover, we did not find any study in the literature review highlighting its action on the *C. albicans* of the acrylic dentures.
2. Brown coloration of the denture resins soaked in other antiseptics like chlorhexidine has not been described with hexetidine. This color can be a major obstacle in the use of soaking solutions as a daily decontamination product.

Our results showed that among the 20 patients of the test group, dentures soaked in hexetidine 0.1%, only 2 registered a total disappearance of *C. albicans* (0 CFU/ml), 16 had reduced number of colonies for more than 80% (3 for more than 60 %), 1 patient went from more than 1,000,000 CFU/ml before soaking to 120000 CFU/ml at D4 sampling and 1 patient had a higher number of colonies at D4 compared to D1.

The effect of hexetidine on *C. albicans* is, therefore, variable but is not null. On the other hand, among the 20 dentures soaked in hexetidine, 4 underwent a very light brownish stain. These four prostheses were all old in age. In our opinion, further studies need to be done in order to confirm this point, which may be due to a factor related to the age of the resin.

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

Conclusion

Hexetidine solution presents moderately good results after soaking dentures for 8 h during 4 consecutive nights. In this respect, it deserves subsequently more research, under various operating protocols to enable an objective study of its fungicide effect, and explain the resin coloring problem.

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