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

Georges Aoun¹, Ibrahim Nasseh², Maria Saadeh³, Antoine Cassia⁴, Antoine Berberi⁵

Contributors:
¹Assistant Professor, Department of Oral Pathology and Diagnosis, School of Dentistry, Lebanese University, Beirut, Lebanon; ²Professor, Department of Oral and Maxillofacial Imaging, School of Dentistry, Lebanese University, Beirut, Lebanon; ³Chief of Clinical Services, Department of Orthodontics, School of Dentistry, Lebanese University, Beirut, Lebanon; ⁴Clinical Associate, Department of Orthodontics and Dentofacial Orthopedics, American University of Beirut, Beirut, Lebanon; ⁵Associate Professor, Department of Oral Pathology and Diagnosis, School of Dentistry, Lebanese University, Beirut, Lebanon; ⁶Professor, Department of Oral and Maxillofacial Surgery, School of Dentistry, Lebanese University, Beirut, Lebanon.

Correspondence:
Dr. Berberi A. Department of Oral and Maxillofacial Surgery, School of Dentistry, Lebanese University, Beirut, Lebanon. Email: anberberi@gmail.com

How to cite the article:
Aoun G, Nasseh I, Saadeh M, Cassia A, Berberi A. Effectiveness of hexetidine 0.1% in eliminating Candida albicans colonizing dentures: A randomized clinical in vivo study. J Int Oral Health 2015;S(1):1-4.

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 morbidity 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, cetapyridinium chloride, chlorhexidine digluconate,⁶,⁷ and hexetidine.⁸

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.⁹ 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.\footnote{11}

We included in the study, patients who were: (1) Confirmed having \textit{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 1\textsuperscript{st} 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 \textit{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. \textit{Candida} count was carried out after 48 h incubation at 37°C in aerobic conditions. \textit{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 \textit{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 \( r = \frac{(a-b)}{a} \), “\( a \)” 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 \textit{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 \( (P = 0.124) \), \textit{C. albicans} count on the palate \( (P = 0.516) \) and on the dentures \( (P = 0.484) \).

When comparing the relative reduction of \textit{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 \( (\text{sum of ranks} = 224) \) (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 \textit{Candida}.\footnote{12} Many types of fungal infections occur in the mouth although the most common is the candidiasis caused by \textit{C. albicans}\footnote{13} colonizing oral cavity in 40-60\% of healthy persons.\footnote{14} Dentures predispose to candidiasis in as many as 65\% of people wearing dentures which produces a microenvironment conducive to the growth of \textit{Candida} with low oxygen, low pH, and an anaerobic environment. This may be due to enhanced adherence of \textit{Candida} to acrylic, reduced saliva flow under the surfaces of the dentures or poor oral hygiene.\footnote{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.\footnote{16} It has been widely accepted that proper routine cleansing of dentures is required to prevent DS and maintain healthy supporting tissues.\footnote{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.\footnote{18} It is well accepted that chemical disinfectants have some advantages over mechanical cleaning such as effective disinfection and ease of use.\footnote{19}

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

### References

1. Figueirah MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C. Denture-related stomatitis: Identification of aetiological and predisposing factors - a large cohort. J Oral Rehabil 2007;34(6):448-55.
2. Zissis A, Yannikakis S, Harrison A. Comparison of denture stomatitis prevalence in 2 population groups. Int J Prosthodont 2006;19(6):621-5.
3. Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011;20(4):251-60.
4. Luc J, Roques C, Frayret MN, Michel G, Ducani M, Vandermey J. In vitro bacteriocidal activity of 5 oral antiseptics against the principal microorganisms implicated in oral disease. J Parodontol 1991;10(4):381-7.
5. Sesma N, Rocha AL, Laganá DC, Costa B, Morimoto S. Effectiveness of denture cleanser associated with microwave disinfection and brushing of complete dentures: In vivo study. Braz Dent J 2013;24(4):357-61.
6. Felton D, Cooper L, Duquim I, Minsley G, Guckes A, Haug S, et al. Evidence-based guidelines for the care and maintenance of complete dentures: A publication of the American College of Prosthodontists. J Prosthodont 2011;20 Suppl 1:S1-12.
7. Pavarina AC, Pizzolatto AC, Machado AL, Vergani CE, Giampaolo ET. An infection control protocol: Effectiveness of immersion solutions to reduce the microbial growth on dental prostheses. J Oral Rehabil 2003;30(5):532-6.
8. Ernst CP, Prockl K, Willershausen B. The effectiveness and side effects of 0.1% and 0.2% chlorhexidine mouthrinses: A clinical study. Quintessence Int 1998;29(7):443-8.
9. Budtz-Jørgensen E, Løe H. Chlorhexidine as a denture disinfectant in the treatment of denture stomatitis. Scand J Dent Res 1972;80(6):457-64.
10. Jones DS, McGovern JG, Woolson AD, Gorman SP.
The effects of hexetidine (Oraldene) on the adherence of Candida albicans to human buccal epithelial cells \textit{in vitro} and \textit{ex vivo} and on \textit{in vitro} morphogenesis. Pharm Res 1997;14(12):1765-71.

11. Newton AV. Denture sore mouth. Br Dent J 1962;112:357-60.

12. McCullough MJ, Savage NW. Oral candidosis and the therapeutic use of antifungal agents in dentistry. Aust Dent J 2005;50 4 Suppl 2:S36-9.

13. Patton LL, Bonito AJ, Shugars DA. A systematic review of the effectiveness of antifungal drugs for the prevention and treatment of oropharyngeal candidiasis in HIV-positive patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92(2):170-9.

14. Berberi A, Nourjeim Z, Aoun G. Epidemiology of oropharyngeal candidiasis in human immunodeficiency virus/acquired immune deficiency syndrome patients and CD4+ counts. J Int Oral Health 2015;7(2):1-4.

15. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002;78(922):455-9.

16. Budtz-Jørgensen E, Mojon P, Banon-Clément JM, Baehni P. Oral candidosis in long-term hospital care: Comparison of edentulous and dentate subjects. Oral Dis 1996;2(4):285-90.

17. Pellizzaro D, Polyzois G, Machado AL, Giampaolo ET, Sanità PV, Vergani CE. Effectiveness of mechanical brushing with different denture cleansing agents in reducing \textit{in vitro} Candida albicans biofilm viability. Braz Dent J 2012;23(5):547-54.

18. Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. J Oral Rehabil 2002;29(3):300-4.

19. Paranhos Hde F, Panzeri H, Lara EH, Candido RC, Ito IY. Capacity of denture plaque/biofilm removal and antimicrobial action of a new denture paste. Braz Dent J 2000;11(2):97-104.

20. Sixou M, Hamel O. Bacteriological criterias to consider during antiseptic prescriptions as mouth wash in odonto-stomatolgy. J Parodontol Implant Oral 2002;21(1):25-41.