Clinical and Histological Findings of Denture Stomatitis as Related to Intraoral Colonization Patterns of *C. albicans*, Salivary Flow, and Dry Mouth

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Abstract

Purpose—Multifactorial etiological factors contribute to denture stomatitis (DS), a type of oral candidiasis; however, unlike other oral candidiasis, DS can occur in a healthy person wearing a denture. In this study, we therefore attempt to explore the association between candida, denture, and mucosal tissue using 1) exfoliative cytology, 2) the candidal levels present in saliva, on mucosal tissues and on denture surfaces, and 3) the salivary flow rate and xerostomic symptoms.

Materials and Methods—A cross-sectional study enrolled 32 edentulous participants, 17 without DS as controls and 15 with DS (Newton's classification type II and III). Participants with systemic or other known oral conditions were excluded. Participants completed a xerostomia questionnaire, and salivary flow rates were measured. Samples of unstimulated whole saliva (UWS) and stimulated whole saliva (SWS) were collected. UWS was used for fungal culturing. Periodic acid-Schiff (PAS) stain and quantitative exfoliative cytology were performed on samples from affected and unaffected mucosa from each participant. Levels of Candida species (*albicans* and non-*albicans*) were determined in salivary samples (expressed as colony-forming units, CFU), as well as from swab samples obtained from denture fitting surfaces, in addition to affected and unaffected mucosa.

Results—There were no significant differences in salivary flow rates, mucosal wetness, or frequency of reported dry mouth comparing participants with and without DS. Exfoliative cytology of mucosal smears demonstrated significantly higher (*P* = 0.02) inflammatory cell counts in DS patients, as compared with smears of healthy denture-wearers. *C. albicans* was significantly

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more prevalent in saliva ($P = 0.03$) and on denture surfaces ($P = 0.002$) of DS participants, whereas mucosal candidal counts and the presence of cytological hyphae did not show significant difference comparing DS to healthy participants.

Conclusions—In this investigation, we presented a unique group of healthy edentulous patients. This population may reflect the general DS population without systemic or other oral diseases. The prominent etiological factor for DS in this population is the presence of candida in denture and saliva. We found that other factors such as saliva flow/xerostomia, fitting of the denture, and the presence of candida in the mucosa, are less important in this population. Therefore, DS treatments in healthy patients should first focus on sanitization of an existing denture and/or fabrication of a new denture.

Keywords

C. *albicans*; denture; hyposalivation; stomatitis; exfoliative cytology

Multiple etiological factors contribute to denture stomatitis (DS).[1-6] These factors include 1) microorganisms such as Candida organisms (in particular *Candida albicans*) and gram-negative anaerobes; 2) impaired salivary flow and salivary gland function; 3) trauma from ill-fitting dentures; 4) poor denture and oral hygiene; and 5) impaired immune response secondary to systemic conditions.[5,7-18] Certain strains of Candida, specifically hyphal-forming *C. albicans* clonal types, are more commonly found in candidal infections in DS patients. These virulent strains are capable of epithelial binding, disruption of epithelial integrity, and invasion.[19-24] Besides Candida, antibiotics have been reported to be effective in some refractory DS cases to provide resolution. This suggests that anaerobic pathogens may potentially play a role in some DS circumstances.[13-16] Thus, Candida and gram-negative anaerobes may function together in the pathogenesis of DS.

Impaired salivary flow or altered salivary protein and inorganic composition, often but not always associated xerostomia, have been suggested to lead to a shift in the oral microbiome composition that favors fungal overgrowth. Note that a patient's report of "dry mouth" does not always reflect the reduction of salivary flow or alteration of salivary composition.[25,26] Salivary secretory IgA level is a critical modulator of microbial aggregation, microbial clearance, and surface adherence.[25,26] Thus, impaired sIgA has been attributed to microbial overgrowth.[27-29] The relation between salivary flow and viscosity has been suggested to also potentially play a role in DS by altering the epithelial resistance to candidal binding and invasion.

DS is often associated with ill-fitting dentures associated with atrophic osseous ridge anatomy.[5-7] It is unclear whether the inflammatory state of DS induces ridge resorption resulting in a loose and easily displaced denture, or whether the trauma associated with the denture (e.g., through poor tissue adaption, clenching or inadequate inter-ridge space) can provide a mechanical stress that induces mucosal inflammation and bone resorption via poor tissue perfusion, necrosis, or trauma. Nonetheless, the denture can serve as a habitat for high-density biofilm, which can harbor high levels of bacteria and yeasts, especially in patients with poor oral hygiene, poor denture hygiene, or wearing dentures overnight.[7,17,18] Thus, the denture itself is believed to serve as both traumatic inducer and a reservoir for triggering a local microbial infection-mediated inflammatory response.

Current and past literature reveals that patients with compromised immunity due to a systemic condition, such as diabetes, are prone to refractory DS as well as other forms of oral candidiasis.[7-12] Overgrowth of Candida in DS is a common finding and can be a precursor to oro-pharyngeal and esophageal candidiasis, which can become a life-threatening disseminating infection among HIV/AIDS patients or in those with other

immunocompromised conditions, especially those associated with T-cell functional deficits.[22-24]

Although several studies have been done to examine these etiologic factors of DS, most focus on only one or a few factors. Moreover, a large body of literature on DS presents results that overlap between DS patients with other forms of oral candidiasis. Note that healthy individuals without dentures will not have any oral candidiasis. On the contrary, healthy denture wearers often present with DS. It is plausible that in the general denture-wearing population, some etiological factors may play a larger role than others. For example, healthy denture wearers would have little problem from systemic and local immunity or from xerostomia or impaired salivary function. The interaction between candida and the denture mediated with normal saliva is perhaps the most prominent DS etiological factor in healthy denture wearers.

To reflect healthy denture wearers with limited influence from systemic conditions and other factors besides DS that contribute to oral candidiasis, we decided to conduct an exploratory cross-sectional investigation. Our goal was to examine the association between clinical signs of DS (measuring the severity of stomatitis with the Newton Classification[30] and the denture fit using the Kapur index[31]) and candidal overgrowth by examining 1) the exfoliative cytology, 2) the candidal levels present in saliva, on mucosal tissues, and on denture surfaces, and 3) salivary flow rate and xerostomic symptoms. We believe that the overall healthy status of the patient population provides a unique cohort for studying the etiology of DS, reflecting the majority of denture-wearing population.

Materials and methods

Study design, participants, and sample size estimates

This was a single-center, case control study design intended to collect biological samples from participants with and without denture stomatitis, specifically with mucosal lesions (Newton's Classification type II or type III).[30] The following biological markers were obtained: 1) gene expression profiles as determined by Affymetrix (Affymetrix Inc., Santa Clara, CA) arrays obtained using tissue biopsy samples, 2) exfoliative cytology was performed on mucosal surfaces to examine for the presence or absence of *C. albicans* infection as determined by periodic acid-Schiff (PAS) cytology, 3) the presence or absence and relative level of *C. albicans* on mucosal and denture surfaces was determined using cultivable methods, 4) tissue and denture surfaces were sampled to measure the presence, absence, and levels of 18 dental biofilm organisms by deoxyribonucleic acid-deoxyribonucleic acid (DNA-DNA) checkerboard and cultured for *C. albicans*, 5) the rate of salivary flow and the levels of selected pro-inflammatory cytokines in saliva were measured and 6) C-reactive protein levels were measured in the serum.

This was an exploratory study, and a targeted sample size of 30 (15 patients per group) was determined to provide 80% power with two-sided alpha = 0.05 significance tests to detect changes in means from continuous variables between diseased and non-diseased patients that are 1.06 times the standard deviation of the variable. The sample size was considered sufficient as effect sizes such as this, or larger, are common for levels of dental biofilm organisms and pro-inflammatory cytokines.[7,17,22] To allow for an 8 to 10% possible drop-out rate, 32 patients were enrolled to ensure that 30 (15 participants per group) completed the study. In this report, we limit the analyses to the clinical findings, the cytology, and the cultivable data.

The study protocol was approved by the UNC Institutional Review Board (IRB), No. 07-2014. A total of 32 edentulous patients were enrolled according to the inclusion and

exclusion criteria listed in Appendix 1. The control group (n = 17) had no signs or symptoms of DS, and the diseased group (n = 15) presented with type II (n = 8) or III (n = 7) DS (Newton's classification).[30] This level of DS includes the moderate (type II) and severe (type III) forms. Representative clinical appearance of cases and controls appear in Figure 1. All enrolled participants completed the two-visit study. For all parameters of interest, there was no apparent gradient progressing from health to type II to type III; therefore, type II and type III remained grouped as a single DS diseased group, as initially intended.

Demographics

The mean age of the 32 participants was 64.8. The healthy group was comprised of 14 women, 3 men, 11 Caucasians, and 6 African Americans. The stomatitis group was comprised of 9 women, 6 men, 9 Caucasians, 3 African Americans, and 3 Asians. There were no significant differences in age, race, or gender in the DS and Healthy groups. Baseline characteristics and Kapur index results are included in Table 1 and show no significant differences between groups.

Clinical evaluation of dentures (Kapur Index)

A qualified examiner (prosthodontist) assessed the fit of the maxillary and mandibular dentures (if present) using the Kapur Index[31] as following:

Retention:

3: Good – maximum resistance to vertical pull and sufficient lateral force.

2: Moderate – moderate resistance to vertical pull and little or no resistance to lateral force.

1: Minimum – slight resistance to vertical pull and little or no resistance to lateral force.

0: No retention – denture displaces itself.

Stability:

2: Sufficient – demonstrates slight or no rocking on its supporting structures under pressure.

1: Some – demonstrates moderate rocking on its supporting structures under pressure.

0: No stability – demonstrates extreme rocking on its supporting structures under pressure.

Because not all of the patients had a mandibular denture, mean values for the total scores of retention and stability for the maxillary denture were calculated.

Clinically poor denture = sum score < 3

Clinically fair denture = sum score 3-4.

Clinically good denture = sum score > 4.

Unpaired T-test was performed to assess statistical difference between means of scores of the maxillary dentures for DS and healthy groups.

Unstimulated saliva collection

Patients were instructed to remove their dentures and refrain from eating, drinking, smoking, brushing their teeth, or chewing gum for 15 minutes prior to salivary collections. All collections were performed between 9:00 and 11:00 AM. Participants were instructed to swallow to clear the mouth of any accumulated saliva, and whole unstimulated saliva was allowed to pool in a sterile polypropylene graduated collection vial for 5 minutes. A fraction

of the sample was sent immediately to the microbiology lab for rapid processing to prevent overgrowth of the Candida species. Samples were aliquoted into Eppendorf tubes, centrifuged for 10 minutes at 3000 g, and the supernatant stored at -80°C for proteomic and cytokine analysis.

Stimulated saliva collection

Patients were instructed to place their dentures back before collection began. Stimulated whole saliva was quantified using a modification of the Saxon test,[32] where each participant was asked to chew on a folded strip of paraffin for 2 minutes after swallowing to clear the mouth of the accumulated saliva. The collected saliva was then quantified as milliliters of saliva generated per minute (ml/min) and recorded on the CRF. Participants found to have a resting (unstimulated) saliva rate of less than 0.01 ml/min and stimulated salivary rate of less than 0.10 ml/min were characterized as having hyposalivation.[33,34]

Xerostomia questionnaire

Patients were asked to complete the xerostomia questionnaire (Appendix 2) after the salivary collection. This questionnaire is derived from the validated questionnaire from the Dental ARIC study as described by Beck et al.[35] ANOVA (items # 3, 13 and 14) and Spearman correlations (items #5-12) by regression analysis were used to analyze relationships between salivary flow rates and patient-reported symptoms of dry mouth.

Mucosal wetness

Patients were instructed to swallow, and sialopaper was placed on the midline of the anterior third of the dorsum of the tongue for 5 seconds. Sialopaper was then transferred to Periotron (Model 6000) (Ora Flow Inc. Plainview, NY) for reading. After reading, the sialopaper was discarded. Measurement was done twice. T-test was used to determine differences between the means of readings comparing groups.

Exfoliative examination

Smears were taken from the affected palatal mucosa and unaffected palatal mucosa for the stomatitis group, and only one sample from the palatal mucosa for the control group. Samples were taken from the buccal vestibular area and dorsum of the tongue as well. Participants with dry mouth (xerostomia) were instructed to rinse with a small amount of water prior to collection of the sample. Selected areas were wiped firmly, using a wooden tongue blade, until a visible accumulation of oral fluids was present. Accumulated samples were transferred to a clean glass slide until a thin coat was visible when the slide was held against light. Slides were then sprayed with Cytofix/Cytoperm (Becton, Dickinson and Company, Franklin Lakes, NJ) with one or two swipes from a distance of about 1 foot and allowed to dry for 10 minutes.

When adequate samples could not be collected using this procedure, the sampling steps outlined above were repeated in a different area of the palatal mucosa, until an adequate sample was collected. These samples were used for cytology and PAS identification of fungal forms. The following scoring system was used for the PAS-cytology assessments:

0 = Inadequate cell sample, 1 = Benign smear, 2 = Bacteria only, 3 = Benign inflammatory smear, 4 = Bacteria plus inflammatory cells, 5 = Fungal spores, 6 = Fungal organisms.

Culture

BBL CultureSwab (Becton, Dickinson and Company) was used to swab the denture and the mucosal surface, with a total of three swabs for the diseased group (from the denture,

mucosal surface of affected area, and unaffected area), and two swabs for the control group (from the denture and the mucosal surface). Samples obtained from swabbing were cultured on Sabouraud Dextrose agar containing quemicetine succinate. Samples were spiral plated to Sabouraud's dextrose plates to obtain a quantitative value of CFU/ml. Samples were also plated on a total aerobic plate to compute the percentage of total aerobic recoverable CFU on a non-selective medium to allow for identification of both *albicans* and non-*albicans* Candida. Candida colonies were counted after 48 hours, and the patients classified according to the number of CFU (colony-forming units) as follows: negative (CFU/ml = 0), carrier (CFU/ml < 400), and positive (CFU/ml > 400). A fraction of the UWS was sampled in Sabouraud Dextrose agar containing quemicetine succinate. Candida colonies were counted after 48 hours, and the patients classified in the same way as for the BBL culture swabs.

Mucosal biopsy

At the end of the appointment, patients were anesthetized, and 4 mm punch biopsies were collected from the palate. In healthy patients, the biopsy was taken from the posterior ridge area. All patients were followed up 1 week post-surgery. In DS participants, two punch biopsies were taken: a diseased sample from the palatal area that was the most severe clinical area, and a second biopsy from a relatively less-affected area of inflammation, sampling the palatal site that was most normal in appearance. All biopsies extended to the periosteum and included a full-thickness mucosal sample from periosteum to keratinized epithelium.

Results

Salivary flow rates

There was no significant difference in the rate of stimulated or unstimulated salivary flow comparing healthy individuals to DS patients (Table 2).

Xerostomia symptoms

There was no significant difference in the frequency of reported dry mouth comparing DS to control patients; however, among all patients there were significant associations between symptomology and flow and wetness measures. A significant correlation was found between unstimulated salivary flow rate and perceived "Rate the dryness of your tongue" ($r^2 = 0.36$, $p = 0.0405$). There was also a significant association between stimulated salivary flow rate and "Rate the level of your thirst" ($r^2 = 0.51$, $p = 0.0028$). All other correlations were not significant and were in the range of (-0.23 to 0.10). For questions #3, 13, and 14 there were no significant associations between the reported symptoms and the flow rate of both unstimulated and stimulated saliva.

Cytology

According to the cytology results of the palatal mucosal swabs (Fig 2, Table 3), it was noticed that type II and type III DS are associated with higher scores reflecting inflammatory cells, bacteria, and fungal morphotypes. Inflammatory cells in the palatal mucosa showed a significant difference ($P = 0.02$) between DS and control participants. It is noteworthy that fungal forms were often found in control participants. There was no statistically significant difference in the cytology of the vestibular and tongue swabs comparing DS type II or type III to health; however, the vestibular area and the tongue as well as the palate had a trend for more prevalent fungal forms in DS patients.

Cultivable *C. albicans* levels in saliva and on denture and mucosal surfaces

In the saliva, *C. albicans* was detected in 80% (12/15) of the DS participants, but only 41.2% (7/17) of the control participants ($p = 0.03$, Chi Square). There was no greater detection rate of C. *albicans* in severe (type III) DS as compared to mild (type II) DS. In the dentures, *C. albicans* was present in 73.2% of dentures sampled from DS participants and only in 11.8% of dentures sampled from healthy individuals ($p = 0.002$), Chi Square. In the mucosal swabs there was no significant difference in *C. albicans* counts between diseased and healthy mucosa ($p = 0.2$). There was a high degree of concordance between the presence of *C. albicans* in the saliva vs. that detectible on the denture ($r^2 = 0.31$, $p = 0.0007$). *C. albicans* prevalence by location comparing saliva vs. healthy mucosa was not significant ($p = 0.059$), nor was it significant when compared to diseased mucosa ($p = 0.79$). Among participants with detectible *C. albicans*, the level (CFU) of *C. albicans* within the saliva tended to be higher among DS participants, compared to healthy controls, but the difference was not statistically significant (Fig 3). When *C. albicans* was present, the mean salivary level of log CFU was 5.03 in DS and 4.4 in health. This difference (0.63) indicates that the mean level was 4.3-fold higher in the saliva of participants with DS when detected (Fig 3). Non-*albicans* Candida was also significantly detected, especially in DS patients. Table 4 shows the prevalence of both *C. albicans* and non-*albicans* in all patients.

Discussion

Role of saliva

In healthy denture wearers without a systemic condition, saliva is known to be an important etiological factor in the development of DS. In this study, there was no difference in the frequency of reported dry mouth, or in the rate of stimulated or unstimulated salivary flow comparing control individuals to DS patients. Note also that most of the participant-reported symptoms of dry mouth did not correlate with saliva flow. Only three questions (out of 14) in the xerostomia questionnaires had any statistical significance. Torres et al[9] found that 67.9% of the individuals with xerostomia were colonized by Candida spp.; however, the difference between these patients and those without xerostomia was not statistically significant. Närhi et al[8] found significantly higher counts of yeasts in individuals with salivary flow rates below normal. According to Pereira-Cenci et al[36] patients with low or impaired salivary flow and/or composition presented higher Candida species counts when compared with saliva from patients with normal salivary flow. Hibino et al[37] reported that both stimulated and unstimulated salivary flow rates of the non-carriers were higher than the carriers, although the difference was not statistically significant.

In their study to determine risk factors associated with oral candidiasis onset and chronic maintenance, Campisi et al[38] found both denture wearing and xerostomia to be local risk factors according to their analysis. The sample size in our study is too small to refute or resolve any of these conflicting findings. Our results, however, suggest that at least in healthy denture wearers, DS can develop with normal saliva flow. One key issue is that the typical assessments of salivary function from a physiological perspective (e.g., salivary flow rate, mucosal wetness, xerostomia symptoms) may not be associated with the antifungal capacity of the saliva. Clearly, future studies intending to examine the role of salivary immunoglobulins (e.g., sIgA) and specific anti-fungal components, such as lactoferrin, histatin-5, lysozyme, and histidine-rich polypeptides in recoverable yeast counts, in addition to physiological assessments, would be informative.

Denture fitting

In this study, the fit of the dentures does not appear to be a strong contributing factor for DS in healthy denture wearers. There was no statistical difference in Kapur index between DS

and control subjects (Table 1); however, it is important to point out that the average Kapur index for the control patients was in the middle of the fair range, whereas the average index for the DS patients was at the cutoff between the fair to poor range. The small sample size limits our interpretation of the contribution of denture fit to DS in healthy patients, and it is possible that this factor may be statistically significant in a larger sample size.

Role of Candida

In this study, *C. albicans* was approximately twice as likely to be present in the saliva of DS patients (80%) as compared to control patients (35.3%); however, there was no clear trend for the CFU levels of salivary *C. albicans* to show a gradient of higher numbers associated with the transition from type II to type III DS. When *C. albicans* was detected in healthy or DS participants, the level within the saliva was found to be 4.3-fold higher among DS participants.

A strong relationship between DS and the presence of Candida in saliva has been reported previously.[39,40] On the other hand, patients with Candida in saliva may not develop DS.[41] This is in agreement with our findings. Since the frequency of detection of *C. albicans* is lower on the denture (11.8%) than in the saliva (41.2%) of healthy patients, it would appear that saliva and/or mucosa represents the reservoir of infectious agent in these healthy individuals and that the denture is less frequently colonized with *C. albicans*. According to Pires et al,[42] the clinical resolution of DS was not related to the levels of Candida in saliva and, furthermore, a decrease of Candida counts in saliva usually is not followed by clinical improvement of DS.

In our study, there was a significant difference in the prevalence of detectible C. *albicans* counts from denture surface between DS and control participants ($p = 0.03$). C. *albicans* was 20.6-fold more likely to be cultured from the denture in DS patients than in healthy ones (i.e., 11/15 vs. 2/17, Table 4). If we examine the levels of *C. albicans* cultured from dentures there was a non-significant trend for higher denture counts among those with DS. Grouping the denture counts for healthy patients into three categories as no growth, [1+, 2+], and [3+, 4+] resulted in a distribution of 41.2%, 47.1%, and 11.7%, respectively. Similarly for denture stomatitis samples the distribution was 26.7%, 40%, and 33.3% ($p = 0.18$), suggesting the trend toward higher denture counts of *C. albicans* in DS participants. The notion that greater numbers of Candida spp. were recovered from smears prepared from the fitting surfaces of the dentures than from those on the palatal mucosa has been known since the 1970s.[43,44] It has been shown that *C. albicans* colonies were recovered more frequently from the tissue fitting surface of the acrylic resin denture than from the corresponding palatal mucosa in DS patients.[40] However, according to Radford et al,[45] it is difficult to attribute the etiology of the condition entirely to the presence of *C. albicans* in denture plaque, as they found in their review that methods of sample collection accounted for individual variation, since palatal imprints yielded 55% of participants with yeast present, denture plaque sampling 80%, and saliva sampling 95%. Interestingly, in our study we found a high degree of concordance between the presence of *C. albicans* in the saliva vs. that detectible on the denture ($r^2 = 0.31$, $p = 0.0007$). It was also noticed that non-albicans species are more prevalent in control patients, especially upon comparing prevalence in the unaffected mucosa in these individuals; however, whether non-albicans presence in patient's saliva or mucosa is associated with any protective effect cannot be concluded from this small cohort.

Exfoliative cytology findings in DS

Reports have been variable on whether the inflammation associated with denture stomatitis is secondary to denture trauma or if it is simply a result of candidal infection. According to

Barbeau et al,[1] the presence of yeasts on the denture in denture-related stomatitis is probably linked to extensive inflammation. Considering the hypothesis that inflammation could be present before Candida colonization, this could explain the variable results in the treatment of denture-related stomatitis with antifungal treatment alone.[1]

According to Edgerton and Levine,[46] stomatitis pathogenesis can occur through two separate mechanisms. They state, "Alterations in the composition of pellicle formed in stomatitis conditions, such as degradation of pellicle components, may directly promote colonization of *C. albicans* on "stomatitis" pellicle. Alternatively, *C. albicans* may be a secondary colonizer or may require bacterial cell products to stimulate adhesion, as has been shown with in vitro studies of *Streptococcus mutans* and C. *albicans*.[47] In this case, altered pellicle deposition in the disease process may initially enhance adhesion of other bacteria, which would subsequently promote adhesion of Candida."[46]

Ritchie et al[48] found that bacteria, leukocytes, and yeast hyphae could be detected in all patients even when cultures were negative. Examination of PAS-stained smears prepared from denture scrapings showed higher number of yeast cells in DS patients by Budtz-Jorgensen et al[13] as well; however, in our study, the presence of hyphae, as determined from the PAS smear from tissue was not pathognomic for disease.

Conclusions

The overall healthy status of the patient population in this study provides a unique cohort for the study of three important DS etiological factors including candida, denture, and saliva. DS in healthy patients appears to have a unique pathogenesis different from other oral candidiasis. We selected participants to limit the influence of systemic conditions and others (e.g., medications) that may compromise immunity and saliva factors. In this study, we confirm that DS is perhaps a result of the denture acting as a reservoir of candidal organism. Normal saliva in healthy patients appears to be the medium for candidal organisms' movement between the denture and tissue, but plays a limited role in DS development. Denture fitting and xerostomic factors are perhaps not primary factors, at least in this same sample size, healthy, denture-wearing population. The results also suggest that unlike other oral candidiasis, there is no association between the fungal presence in the tissue and the clinical symptoms of denture stomatitis. Therefore, treatments for DS should first focus on sanitization of an existing denture or fabrication of a new denture, rather than antifungal treatment in healthy denture wearers.

Acknowledgments

Appendix 1

Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1 At least 45 years of age	1 Less than 45 years of age.

Inclusion criteria	Exclusion criteria
2 Men or women without menses for 12 consecutive months or who have had a complete hysterectomy	**2** Have chronic disease with oral manifestations other than denture/mucosal stomatitis.
3 Wear complete maxillary denture (overdentures, implant- or tooth-retained dentures acceptable) without daily use of denture adhesive.	**3** Have gross oral pathology.
4 Must have read, understood, and signed an informed consent form.	**4** Have overt denture abrasion associated with symptoms.
5 Must understand and be willing to comply with all study procedures and restrictions	**5** Participants with clinically significant organic diseases, including impaired renal function, bleeding disorder, or any condition requiring antibiotic pre-medication for dental visits
6 Must be in good general health; diabetics included	**6** Participants with active infectious diseases such as hepatitis, HIV, or tuberculosis.
7 Must have type II or type III denture stomatitis for denture stomatitis group	**7** Participants who are immunosuppressed because of medications or condition.
8 Must have no signs of denture stomatitis for control group	**8** Participants who have used antibiotics or antifungals for any medical or dental condition within 1 month prior to screening.
	9 Participants using ongoing medications initiated less than 3 months prior to enrollment.
	10 Participants with a known or suspected intolerance to local oral anesthesia.
	11 Participants who have participated in another clinical study or have taken an investigational drug within 30 days of screening.
	12 Participants who have used tobacco products within 6 months of screening.
	13 Employees of the sponsor or the investigator or members of their immediate family.
	14 Participants who have previously participated in this study.
	15 Post-menopausal women on hormone replacement therapy.

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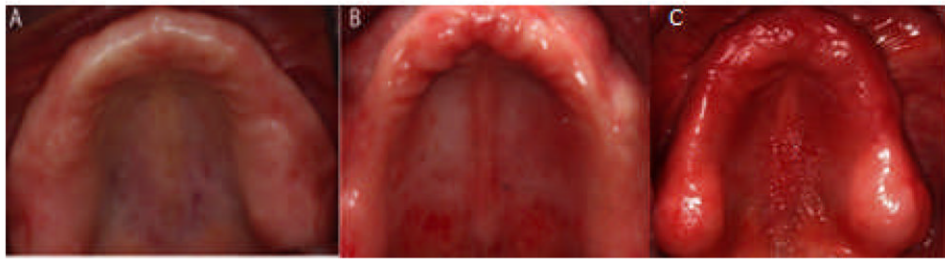
Figure 1.
Clinical photographs of (A) a control patient, (B) Newton classification II stomatitis, and (C) Newton classification III stomatitis.
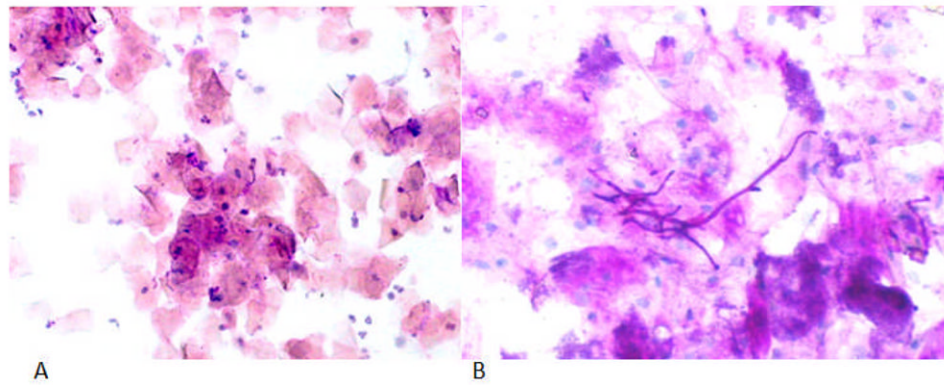
Figure 2.
PAS exfoliative cytology representative slides. (A) a healthy control benign smear; oral cytologic smear of palatal mucosa showing typical squamous cells and scattered chronic inflammatory cells. (10 X), (B) DS fungal hyphae; oral cytologic smear from palatal mucosa showing candidal hyphae. (40X).
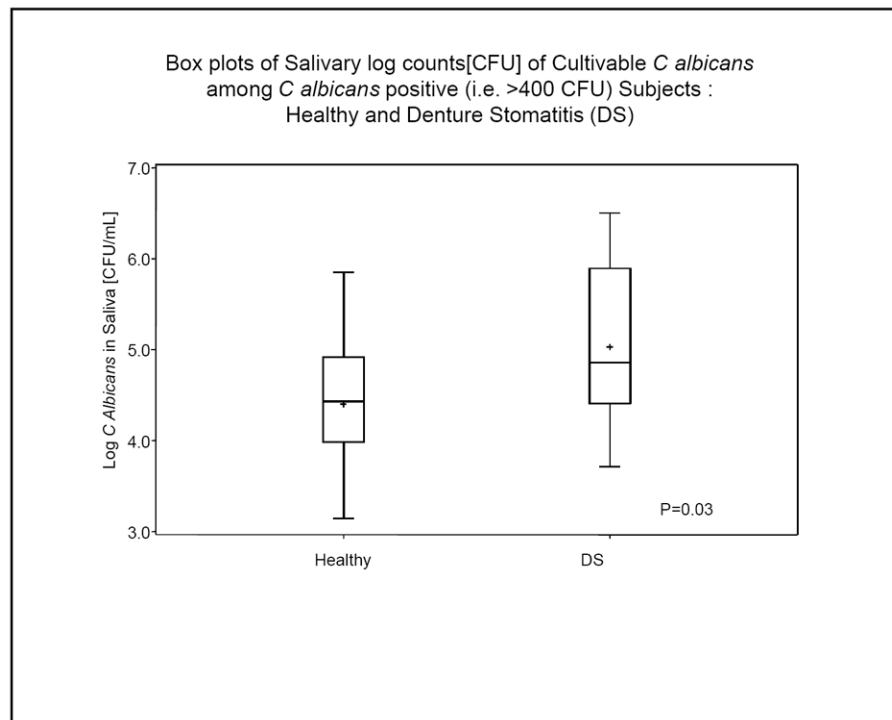
Figure 3.
Mean log *C. albicans* counts in saliva among DS and control participants. The boxplot indicates the log CFU *C albicans* / mL of saliva. The plot shows median, upper, and lower 25th percentile range (box boundaries) and range as outer markings. The plus sign shows the mean log values.

Table 1

Demographics and baseline characteristics of the participants

	Control (n = 17)	DS (n = 15)
Age (mean ± SD)	66.2 ± 9.7	63.2 ± 8.83
Gender		
Male	3 (17.6%)	6 (40%)
Female	14 (82.4%)	9 (60%)
Race		
Caucasian	11 (64.7%)	9 (60%)
African American	6 (35.3%)	3 (20%)
Asian		3 (20%)
Kapur Index of max. Denture (mean ± SD)	3.56 ± 1.46	3.00 ± 1.57

Table 2

Flow rate of unstimulated and stimulated whole saliva in control and DS participants

Participants	Salivary flow (ml/min) UWS (mean ± SD)	SWS (mean ± SD)
Control (n = 17)	0.5 ± 0.23	1.43 ± 0.57
DS type II (n = 8)	0.5 ± 0.15	1.14 ± 0.43
DS type III (n = 7)	0.55 ± 0.23	1.34 ± 0.57
P value (ANOVA)	0.84	0.46
DS combined (n = 15)	0.53 ± 0.19	1.23 ± 0.5
P value (unpaired T-test)	0.71	0.31

- UWS: unstimulated whole saliva collected for 5 minutes.

- SWS: stimulated whole saliva collected by chewing folded strip of paraffin for 2 minutes.

- Hyposalivation: unstimulated saliva rate of less than 0.01 ml/min, and stimulated saliva rate of less than 0.10 ml/min.

Table 3

PAS exfoliative cytology results for palatal and vestibular area and tongue

	Palatal area			Vestibular area and tongue		
	Healthy	Diseased	p-value	Healthy	Diseased	p-value
Adequate (Yes)	12 (44.4%)	15 (55.6%)		17 (53.1%)	15 (46.9%)	
No	5 (100.0%)	0 (0.0%)	0.02	..	..	..
Bacteria (Yes)	6 (40.0%)	9 (60.0%)		12 (46.2%)	14 (53.9%)	
No	11 (64.7%)	6 (35.3%)	0.16	5 (83.3%)	1 (16.7%)	0.10
Inflammatory cells (Yes)	0 (0.0%)	4 (100.0%)		3 (37.5%)	5 (62.5%)	
No	17 (60.7%)	11 (39.3%)	0.02 *	14 (58.3%)	10 (41.7%)	0.31
Fungal spores (Yes)	5 (35.7%)	9 (64.3%)		12 (46.2%)	14 (53.9%)	
No	12 (66.7%)	6 (33.3%)	0.08	5 (83.3%)	1 (16.7%)	0.10
Fungal hyphae (Yes)	4 (40.0%)	6 (60.0%)		7 (43.8%)	9 (56.3%)	
No	13 (59.1%)	9 (40.9%)	0.32	10 (62.5%)	6 (37.5%)	0.29
Atypical cells (Yes)	0 (0.0%)	1 (100.0%)		..	..	
No	17 (54.8%)	14 (45.2%)	0.28	17 (53.1%)	15 (46.9%)	..
Score						
Inadequate cell sample	6 (100.0%)	0 (0.0%)		..	..	
Benign smear	4 (57.1%)	3 (42.9%)		4 (100.0%)	0 (0.0%)	
Bacteria only	2 (50.0%)	2 (50.0%)		1 (50.0%)	1 (50.0%)	
Benign inflammatory cells	0 (0.0%)	1 (100.0%)		..	..	
Bacteria plus inflammatory cells	..	..		..	..	
Fungal spores	1 (25.0%)	3 (75.0%)		5 (50.0%)	5 (50.0%)	
Fungal organisms	4 (40.0%)	6 (60.0%)	0.13	7 (46.6%)	8 (53.3%)	0.28
Score dichotomized (fungal)	5 (35.7%)	9 (64.3%)		12 (48.0%)	13 (52.0%)	
No fungal	12 (66.7%)	6 (33.3%)	0.08	5 (83.3%)	1 (16.7%)	0.12

*
Statistically significant ($P < 0.05$)

Table 4

Prevalence of detectable *C. Albicans* and non-*albicans* in saliva, denture, and mucosal surfaces for control and DS participants. The table indicates the number of patients positive for Candida [*albicans* and *non-albicans*] and the percent positive within the disease category. The p-values are chi-square statistics between the control and the DS groups.

	Saliva (N = 32)		Denture (N = 32)		Unaffected mucosa (N = 32)		Affected mucosa (N = 15)	
	C. albicans	Non-albicans	C. albicans	Non-albicans	C. albicans	Non-albicans	C. albicans	Non-albicans
Control (N = 17)	7 (41.2%)	7 (41.2%)	2 (11.8%)	8 (47.1%)	1 (5.9%)	7 (41.18%)		
DS type II (N = 8)	7 (87.5%)	0 (0.00)	6 (75.0%)	0 (0.00%)	2 (25.0%)	2 (25.0%)	2 (25.0%)	3 (37.5%)
DS type III (N = 7)	5 (71.4%)	0 (0.00)	5 (71.4%)	2 (28.6%)	2 (28.6%)	4 (57.1%)	4 (57.1%)	3 (42.9%)
Total (N,%)	19 (59.4%)	7 (21.9%)	13 (40.6%)	10 (31.3%)	5 (15.6%)	13 (40.6%)	6 (40.0%)	6 (40.0%)
P values	0.03*	0.02*	0.002*	0.06*	0.27	0.45	0.20	0.83

*
Statistically significant ($P < 0.05$)