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EVALUATION OF DENTURE-RELATED FACTORS PREDISPOSING TO DENTURE STOMATITIS IN A LEBANESE POPULATION

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ABSTRACT

Background: Denture stomatitis is a common fungal oral infection of multifactorial etiology. Denture-related factors such as denture's age, hygiene, night wearing and colonization by *Candida albicans* are known to predispose to denture stomatitis. The aim of this study was to evaluate these factors and their relationship with the occurrence of denture stomatitis in a Lebanese population. **Material and methods:** Sixty patients (40 women, 20 men; age range 40-80 years) showing clinical evidence of denture-related stomatitis were selected for this study. Swab samples from the palate and the palatal surfaces of the upper dentures of these patients were collected and examined mycologically. Denture's age, hygiene, night wearing and colonization by *Candida albicans* were evaluated and analyzed statistically. **Results:** The statistical analysis showed a significant role for denture's hygiene, night wearing, and colonization by *Candida albicans* in the installation of the denture stomatitis. Patient's gender and age, as well as the denture's age, were not significant predictors of the disease. **Conclusion:** In a sample of Lebanese population, poor denture hygiene, continuous denture use, and *Candida* denture colonization are important etiological factors that contribute to denture stomatitis.

Key words: Denture, denture stomatitis, Lebanese population.

1. INTRODUCTION

Denture stomatitis (DS) designates the inflammation of the oral mucosa mostly the palatal, underlying a denture (1).

It is found in around 50% of edentulous patients wearing dentures and it is more frequent in women than in men (2).

In 1962, Newton classified DS into 3 types on a clinical basis (3):

- Type one: a focal inflammation which may indicate the early stage of the disease.
- Type two: a generalized inflammation characterized by a diffuse erythema of the mucosa covered by the denture.
- Type three: an inflammatory papillary hyperplasia.

Type two is the most common between the 3 types of DS (4).

Predisposing factors to DS are usually divided into local and systemic (5-7).

Diabetes mellitus and conditions of nutritional and immunity deficiencies are among the systemic factors.

As for the local factors, the most commonly stated are connected to the denture's age, poor hygiene, microbial colonization, and continuous wearing.

The aim of this study was to evaluate the correlation between DS and these local factors and to determine its occurrence in a Lebanese population.

2. MATERIALS AND METHODS

This study was conducted in accordance with the Helsinki agreement for research on humans, and its design was approved by the Institutional Review Board of the School of Dentistry, Lebanese University; Beirut, Lebanon. Written informed consent was obtained from all participants.

Were included in this study, patients:

- Aged between 40 and 80 years old.
- Showing clinical signs of DS; Newton type two.
- Not having any systemic disease known to be predisposing DS such as diabetes, nutritional deficiencies, etc...
- Not taking any medication that might affect the oral bacterial flora.
- Wearing the maxillary complete denture for more than a year.

Sixty full acrylic maxillary denture-wearing patients (40 women and 20 men) meeting the inclusion criteria were

selected and divided into 2 categories:

- Age between 45 and 65 years old.
- Age between 66 and 85 years old.

A quantitative microbiological measurement was performed from the selected patient’s palatal mucosa and the fitting side of their dentures.

The same investigator carried out the microbiological procedures. The BBL Culture Swabs, sterile devices for collecting and transporting microbiological specimens (Amies, Stuart, and Agar gel), from [Becton-Dickinson (New Jersey, USA) Microbiology System] were used.

Culture of swabs was done in Sabouraud’s dextrose agar (dextrose 40 g/l, peptone 10 g/l, and agar 20 g/l), chloramphenicol 0.5 g/l, and actidione 0.5g/l. The incubation time was set to 48 hours at 37°C in aerobic conditions.

To differentiate *Candida albicans* from other species, 0.5 ml of animal serum was added to provoke the filaments production.

Candida albicans colony counts expressed in colony-forming unit (CFU)/ml collected from the dentures surfaces and the palates were noted using a classification from 5 categories.

- Category 1: ≤ 1000 CFU/ml.
- Category 2: >1000 ≤ 5000 CFU/ml.
- Category 3: >5000 ≤ 10000 CFU/ml.
- Category 4: > 10000 ≤ 15000 CFU/ml.
- Category 5: > 15000 CFU/ml.

Additionally, the denture hygiene was visually evaluated using a plaque disclosing product as proposed by Budtz-Jørgensen and Bertram (8), and classified into 3 grades:

- Grade 1: good (free of stains).
- Grade 2: satisfactory (less than 50% of the denture stained).
- Grade 3: poor (over 50% of the denture stained).

The age of the dentures was also noted and classified into 3 ranks:

- Rank 1: between 1 and 4 years.
- Rank 2: between 4 and 8 years.
- Rank 3: more than 8 years.

Finally, concerning the night wearing of the denture, it was classified as following:

- Status 1: no night wearing (removal of the denture during the night).
- Status 2: regular night wearing (continuous use of the denture).
- Status 3: irregular night wearing.

Descriptive statistics for patient age and gender; denture age, hygiene and night wearing in addition to *Candida albicans* colonization of the palate and denture were calculated and percent distribution, means, and standard deviations were reported. Two outcomes were selected for bivariate and multivariate explorations: *Candida* denture colonization and *Candida* palate colonization. Bivariate associations between patient and denture predisposing factors were assessed with each of the two outcomes using simple linear regressions. *P* values were reported for the associations with the overall variable (e.g. denture hygiene) rather than separate associations with each subcategory (e.g. good, satisfactory and poor). Variables associated with either outcome at a significance level of < 0.20 were included in the multivariate

analysis which was conducted using multiple linear regressions.

For all variables included in the final multivariate models, coefficients of association, standard errors, two-sided *p*-values and 95% confidence intervals (CI) were reported in addition to *F* probabilities and adjusted *R*² values. The IBM® SPSS® statistics 20.0 statistical package and Stata/SE™ 11.1 data analysis and statistical software were used to carry out all statistical analyses. Statistical significance was set at 0.05.

3. RESULTS

Descriptive Statistics

The sample consisted predominantly of females (66.7%), over 65 years of age (Table 1). Only a minority displayed good oral hygiene (11.7%). Ninety percent of the examined population was equally divided between those with dentures aged 1-4 years and those with dentures aged 4-8 years; only 10% wearing the same denture for over 8 years. A significant proportion adhered to not wearing their dentures at night (41.7%) but an equal proportion was also regular night-wearers.

| Variable | n | % |
|---------------------------------------|----|------|
| Age (years) | | |
| 46-65 | 12 | 20.0 |
| 66-85 | 48 | 80.0 |
| Gender | | |
| Males | 20 | 33.3 |
| Females | 40 | 66.7 |
| Denture hygiene | | |
| Good | 7 | 11.7 |
| Satisfactory | 19 | 31.6 |
| Poor | 34 | 56.7 |
| Denture age (years) | | |
| 1 - 4 | 27 | 45.0 |
| 4 - 8 | 27 | 45.0 |
| > 8 | 6 | 10.0 |
| Denture night wearing | | |
| Never | 25 | 41.7 |
| Regularly | 25 | 41.7 |
| Irregularly | 10 | 16.6 |
| Candida denture colonization (CFU/ml) | | |
| ≤1,000 | 24 | 40.0 |
| 1,001-5,000 | 19 | 31.7 |
| 5,001-10,000 | 5 | 8.3 |
| 10,001-15,0000 | 6 | 10.0 |
| >15,000 | 6 | 10.0 |
| Candida palate colonization (CFU/ml) | | |
| ≤1,000 | 41 | 68.3 |
| 1,001-5,000 | 12 | 20.0 |
| 5,001-10,000 | 4 | 6.7 |
| 10,001-15,0000 | 2 | 3.3 |
| >15,000 | 1 | 1.7 |

Table 1. Percent distribution of sample background characteristics and exposure and outcome variables (n = 60). Notes: CFU/ml = colony-forming units/milliliter

| Exposure Variable | Candida denture colonization | | P value [†] |
|-----------------------|------------------------------|-----------------------|----------------------|
| | n (%) | Mean (SD) | |
| Age (years) | | | |
| 46-65 | 12 (20.0) | 1,203.5 (1,790.9) | 0.029 [†] |
| 66-85 | 48 (80.0) | 128,236.0 (333,007.0) | |
| Gender | | | |
| Males | 20 (33.3) | 53,017.5 (222,932.9) | 0.370 |
| Females | 40 (66.7) | 127,735.6 (333,906.0) | |
| Denture hygiene | | | |
| Good | 7 (11.7) | 65.7 (34.1) | |
| Satisfactory | 19 (31.7) | 634.8 (900.2) | 0.069 [†] |
| Poor | 34 (56.6) | 181,095.6 (384,798.9) | |
| Denture age (years) | | | |
| 1 - 4 | 27 (45.0) | 1,131.9 (1,312.2) | |
| 4 - 8 | 27 (45.0) | 225,699.6 (421,781.9) | 0.015 [†] |
| > 8 | 6 (10.0) | 7,553.3 (5,978.9) | |
| Denture night wearing | | | |
| Never | 25 (41.7) | 41,437.6 (199,718.1) | |
| Regularly | 25 (41.7) | 164,072.1 (372,380.1) | 0.362 |
| Irregularly | 10 (16.6) | 103,203.0 (315,111.9) | |

Table 2. Bivariate associations between exposure variables and Candida denture colonization (n = 60). Notes: CFU/ml = colony-forming units/milliliter, † p-value < 0.2 and included in multivariate analysis, † P value for entire model reported instead of p values for specific subcategories (Prob > F), *Statistically significant at p < 0.05; **statistically significant at p < 0.01

The majority of the assessed sample exhibited *Candida* palate colonization of no more than 1,000 CFU/ml (68.3%) whereas in terms of *Candida* denture colonization the bulk of the sample (71.17%) exhibited *Candida* concentrations of ≤ 5,000 CFU/ml (40% with ≤ 1,000 and 31% between 1,000 and 5,000). On average, denture colonization with *Candida* was consistently higher than palate colonization except in the subgroup of patients with ≤1,000 FCU/ml in either palate or denture. One in 5 patients exhibited *Candida* concentrations > 10,000 CFU/ml in their dentures compared to 1 in 20 patients showing the same *Candida* concentrations on their palatal mucosa. Similarly, larger proportions of the sample exhibited *Candida* concentrations of between 1,000 and 5,000 and between 5,000 and 10,000 in their dentures than in their palatal mucosa. The relative distributions of *Candida* colonization in dentures compared to in palates suggests that, on average, higher concentrations of *Candida* colonies are present in dentures compared to those present on the palatal mucosa in patients wearing maxillary dentures.

Factors predisposing to denture colonization with Candida albicans

When assessing the bivariate associations between predisposing factors and *Candida* denture colonization, patient age and denture age both showed a significant relationship (p = 0.029 and 0.015, respectively) (Table 2). However, when all variables with bivariate associations of p < 0.20 were modeled (age, denture hygiene and denture age), none of the variables significantly predicted denture colonization with *Candida albicans* (model not shown). Among the predictors assessed in this study only denture hygiene significantly predicted denture colonization with *Candida albicans* (Table

| Exposure Variable | Candida palate colonization | | P value [†] |
|---------------------------------------|-----------------------------|-------------------|----------------------|
| | n (%) | Mean (SD) | |
| Age (years) | | | |
| 46-65 | 12 (20.0) | 1,059.2 (2,540.4) | 0.079 [†] |
| 66-85 | 48 (80.0) | 2,041.6 (3,954.5) | |
| Gender | | | |
| Males | 20 (33.3) | 1,313.0 (2,727.5) | 0.438 |
| Females | 40 (66.7) | 2,111.2 (4,130.5) | |
| Denture hygiene | | | |
| Good | 7 (11.7) | 98.6 (113.8) | |
| Satisfactory | 19 (31.7) | 798.4 (2,044.7) | 0.070 [†] |
| Poor | 34 (56.6) | 2,789.7 (4,497.3) | |
| Denture age (years) | | | |
| 1 - 4 | 27 (45.0) | 243.7 (414.7) | |
| 4 - 8 | 27 (45.0) | 1,812.2 (2,780.1) | <0.001 ^{**} |
| > 8 | 6 (10.0) | 9,200.0 (6,572.7) | |
| Denture night wearing | | | |
| Never | 25 (41.7) | 71.2 (78.8) | |
| Regularly | 25 (41.7) | 4,230.0 (4,876.7) | <0.001 ^{**} |
| Irregularly | 10 (16.6) | 317.8 (133.1) | |
| Candida denture colonization (CFU/ml) | | | |
| ≤1,000 | 24 (40.0) | 606.67 (1,829.6) | <0.001 ^{**} |
| 1,001-5,000 | 19 (31.7) | 852.53 (2,158.7) | |
| 5,001-10,000 | 5 (8.3) | 2,814.0 (3,588.8) | |
| 10,001-15,0000 | 6 (10.0) | 9,186.7 (6,490.7) | |
| >15,000 | 6 (10.0) | 1,793.3 (3,717.3) | |

Table 3. Bivariate associations between exposure variables and denture stomatitis as indicated by Candida palate colonization (n = 60), Notes: CFU/ml = colony-forming units/milliliter, † p-value < 0.2 and included in multivariate analysis, † P value for entire model reported instead of p values for specific subcategories (Prob > F), Statistically significant at p < 0.05; **statistically significant at p < 0.01

4). In patients of the same age, those with satisfactory oral hygiene would present with 177,993.4 more *Candida albicans* colonies than those with good oral hygiene (p = 0.033). According to the model, those with poor oral hygiene would not differ significantly from those with good hygiene, controlling for the effect of age. It is worth noting that the attempt to control for gender in the same model resulted in a non-significant association between gender and *Candida* denture colonization (controlling for age and gender hygiene), did not significantly alter any of the associations between the remaining variables and the outcome and rather decreased the model's adjusted R² (model not shown). Consequently, it was decided to remove gender from the final model. Despite the significance of the final model (Prob > F = 0.011), the variation in *Candida* denture colonization explained by the variables age and denture hygiene is only 13.6% (Adjusted R² = 0.136).

Factors predisposing to DS

At the bivariate level, denture age, denture night wearing and *Candida* denture colonization were significantly associated with *Candida* palate colonization and were therefore included in the multivariate analysis (p ≤ 0.001) (Table 3). Denture hygiene and age were additionally incorporated into the multivariate regression because they displayed as-

sociations with $p < 0.20$ at the bivariate level (Table 5). In the final multivariate model, when controlling for the effects of patient age and denture age, only denture hygiene, denture night wearing and *Candida* denture colonization all significantly predict the colonization of palatal mucosa with *Candida albicans*. When all other variables are constant, patients with poor oral hygiene display an increase of 4,938.0 *Candida albicans* colonies in the palate compared to those with good oral hygiene ($p = 0.001$). Those who regularly wear their denture to bed would display 1,947.7 more *Candida albicans* colonies in the palate than patients who adhere to never going to bed wearing their denture, controlling for the remaining variables ($p = 0.015$). Finally, the presence of large numbers of *Candida albicans* colonies in the maxillary denture, specifically between 10,001 and 15,000 colonies, predict a concomitant increase in the number of *Candida albicans* colonies in the palate by 5,069.2 colonies ($p = 0.004$). Similar to denture colonization with *Candida albicans*, gender did not predict *Candida* palate colonization when added to the selected model and rather reduced the adjusted R^2 and was therefore removed from the final model (model containing gender not shown). The final regression presented (Table 5) explains 62.3% of the variability exhibited in *Candida* palate colonization by our sample of patient (adjusted $R^2 = 0.623$, $\text{Prob} > F \leq 0.001$).

4. DISCUSSION

It has been widely admitted that both systemic and local predisposing factors are behind the etiology of DS (1, 2, 9, 10). Current common belief suggests that interaction of a multitude of these factors exist in the pathogenesis of the disease (11).

Candida albicans, among other species of fungi colonizing dentures, has been proven to be an important factor contributing to DS (8, 12, 13). These opportunistic microorganisms are found in biofilm extremely adherent to the base material of the dentures especially within the micro-fissures and cracks when they exist (14). Under the influence of various predisposing factors, *Candida albicans* undergo structural changes to become pathogenic; thus contributing to the disease onset.

In this study, conducted on a sample of Lebanese adults, four denture-related factors known as predisposing to DS

(denture's age, hygiene, night wearing, and colonization by *Candida albicans*) were assessed. The results showed a statistically significant relationship between DS and the evaluated factors except for the denture's age which was found irrelevant.

The denture age:

Traditionally, the denture age is thought to be a DS predisposing factor. This was confirmed by the studies of Bilhan *et al.* (15), Kossioni (16), Navabi *et al.* (17), and Sada-mori *et al.* (18). In this study, the denture age was not found connected to DS (Rank 2: $p = 0.590$, and rank 3: $p = 0.885$)

| Associated variables | Coef. | Std. Err. | 95% CI | p value |
|-------------------------|---|-----------|----------------------------|---------|
| | Candida denture colonization (CFU/ml) ‡ | | | |
| Constant | -537,291.0 | 325,056.1 | [-1,188,457.0 ; 113,874.6] | 0.104 |
| Age | 8009.6 | 4,768.4 | [-1,542.7 ; 17,561.9] | 0.099 |
| Denture hygiene (Good) | | | | |
| Satisfactory | 177,993.4 | 81,292.7 | [15,324.8 ; 340,662.0] | 0.033* |
| Poor | -65,220.0 | 133,559.1 | [-332,771.1 ; 202,331.1] | 0.627 |
| Adjusted R ² | 0.136 | | | |
| Prob > F | 0.011* | | | |

Table 4. Multivariate analysis showing associations between exposure variables and *Candida* denture colonization (n=60). Notes: CFU/ml = colony-forming units/milliliter; Coef. = regression coefficient; Std. Err. = standard error; Age and Denture age recorded in years. (Base): refers to the base outcome to which all other categories are compared. *Statistically significant at $p < 0.05$; **statistically significant at $p < 0.01$

| Associated variables | Coef. | Std. Err. | 95 % CI | p value |
|---|--------------------------------------|-----------|----------------------|---------|
| | Candida palate colonization (CFU/ml) | | | |
| Constant | -1,062.7 | 2,829.6 | [-6,752.0 ; 4,626.5] | 0.709 |
| Age | 14.7 | 43.3 | [-72.4 ; 101.7] | 0.736 |
| Denture hygiene (Good) | | | | |
| Satisfactory | 486.4 | 857.6 | [-1,237.8 ; 2,210.6] | 0.573 |
| Poor | 4,938.0 | 1409.1 | [2,104.9 ; 7,771.1] | 0.001** |
| Denture age (1-4) | | | | |
| 4 - 8 | -629.2 | 1,159.3 | [-2,960.3 ; 1,701.8] | 0.590 |
| > 8 | -245.4 | 1,691.5 | [-3,645.5 ; 3,155.7] | 0.885 |
| Denture night wearing (Never) | | | | |
| Regularly | 1,947.7 | 768.7 | [402.1 ; 3,493.3] | 0.015* |
| Irregularly | -96.4 | 996.8 | [-2,100.6 ; 1,907.8] | 0.923 |
| Candida denture colonization ($\leq 1,000$) | | | | |
| 1,001-5,000 | 394.6 | 1,251.5 | [-2,121.7 ; 2,910.9] | 0.754 |
| 5,001-10,000 | 591.6 | 1,690.4 | [-2,807.2 ; 3,990.3] | 0.728 |
| 10,001-15,000 | 5,069.2 | 1,688.9 | [1,673.6 ; 8,464.9] | 0.004** |
| > 15,000 | 193.5 | 1,650.2 | [-3,124.4 ; 3,511.4] | 0.907 |
| Adjusted R ² | 0.623 | | | |
| Prob > F | ≤ 0.001 ** | | | |

Table 5. Multivariate analysis showing associations between exposure variables and *Candida* palate colonization (n=60) Notes: *Candida* denture colonization measured in CFU/ml = colony-forming units/milliliter; Coef. = regression coefficient; Std. Err. = standard error; Age and Denture age recorded in years. (Base): refers to the base outcome to which all other categories are compared *Statistically significant at $p < 0.05$; **statistically significant at $p < 0.01$

(Table 5), finding that was consistent with the ones of Mravak-Stipetic *et al.* (11), Naik *et al.* (19), Sahebamee *et al.* (20), and Marinoski *et al.* (21). Despite this negative result, in this sample, the connection between the age of the denture and its contamination by *Candida albicans* was statically significant in bivariate association ($p = 0.015$) (Table 2). This can corroborate the results of many researchers' studies which confirmed that the age of the denture is indirectly linked to DS due to the possibility of the ill-fitting and the increased surface roughness of the denture occurring over time leading to the biofilm/plaque accumulation and consequently to the installation of DS (11, 19, 22).

The denture night wearing:

In our study, night wearing, or continuous use of the denture was seen to be linked to DS ($p = 0.015$) (Table 5). This positive finding supports the ones of many researchers who conducted similar studies in different populations (15, 16, 17, 20). In the literature, continuously wearing the denture was reported to be strongly associated with DS. This was attributed to the fact that putting the oral mucosa in permanent contact with the base of the denture reduces the protective effect of the saliva and prevents proper oxygenation of the mucosa; thus resulting in a decrease in its resistance against mechanical and microbiological aggressions (10, 23).

The denture hygiene:

According to many researchers, the poor denture hygiene stimulates the installation of DS. It is believed that the lack of hygiene induces the plaque/biofilm accumulation, and therefore the bacteria and fungi growth on the palatal denture surface turning the mucosa vulnerable to infection like DS (11, 18, 21, 24). In the present study, the denture hygiene is considered statically significant as DS predisposing factor ($p = 0.001$) (Table 5).

The denture colonization by Candida albicans:

In our study, the third variable, (along with denture hygiene and night wearing), which significantly predicts the installation of DS is *Candida* denture colonization. However, this effect appears to be specific to large numbers of colonies contaminating the fitting surface of the denture ($p = 0.004$) (Table 5). Moreover, the number of *Candida* colonies contaminating the dentures was found higher compared to those colonizing the palatal mucosa. This finding corroborates that of Nanetti *et al.* (22), who found in a randomized study conducted on fifty-two maxillary denture wearers with DS, that the incidence of *Candida albicans* in the mucosa is significantly lesser than that in the denture. As for the age and gender of the patient, they have not been statistically correlated to DS. Finally, our study aiming to evaluate the denture-related factors and their effect on DS, in a Lebanese population, is not without limitations. Because of the limited number of patients assessed and the difference between the female/male samples, definite conclusions must be delayed until future research validates our results.

5. CONCLUSION

A combination of different factors appears to be responsible for the denture stomatitis. According to the present study, the continuous use of the denture, its hygiene, as well as the denture colonization by *Candida* particularly *Candida albicans* are among these factors. On the other hand, the age

and gender of the patient, and the denture age were irrelevant predictors of the disease.

- Conflict of interest: none declared.

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