

Relationships of Candida with Frequency of Brushing, Age and Smoking in Patients with Removable Dental Prosthesis: A Quantitative Study

Alan N. Ghalib¹, Sarhang S. Gul², Jwan F. Abdulkareem³

Abstract

Objective: Although progress has been made to reduce opportunistic infection of fungi in the oral cavity, the prevalence of denture stomatitis associated with Candida has increased. The purpose of this study was to evaluate the effect of denture cleaning frequencies, age and smoking habit on the levels of Candida species in the saliva of patients wearing removable partial or complete dentures.

Methods: Subjects wearing removable partial or complete dentures were recruited. A questioner was used to collect information on oral hygiene habits and smoking. Saliva samples were collected by oral rinse technique in a sterile container and cultured in duplicate Sabouraud Dextrose Agar. The numbers of colonies were determined by aCOLyte colony counter and the number expressed as a colony forming unit (CFU). The CFU and clinical data were analyzed for correlation and the Kruskal-Wallis test was used to determine statistically significant differences.

Results: Among 99 subjects recruited, 47 were wearing complete dentures and 28 were smokers. The brushing frequencies were once (37 subjects), twice (39 subjects) and three times (23 subjects) per day. CFU was significantly higher in partial denture wearers than complete denture wearers and the brushing frequencies significantly correlated with CFU ($r = -0.85$, $P = 0.001$). There was no statistically significant difference in CFU between smokers and non-smokers and no correlation of CFU with age was found.

Conclusions: This quantitative study has suggested that there are statistically significant differences in the levels of Candida in the saliva of subjects with different brushing frequencies and wearing a different type of denture. However, no statistically significant difference was noticed between smokers and non-smokers, and there was no significant correlation between CFU and age.

Keywords: Levels of Candida, Saliva, Denture wearer, Cleaning frequencies.

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1. Department of Microbiology, College of Medicine, University of Sulaimani, Sulaimani, Iraq.
2. Department of Periodontics, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.
3. Department of Prosthodontics, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.

* Corresponding author: alan.ghalib@univsul.edu.iq

Introduction

Candida is a harmless commensal in the oral cavity with a prevalence of 25-50% in healthy subjects⁽¹⁾ and can cause disease when the relation between host and microorganisms is disrupted⁽²⁾. Several local and systemic risk factors are related to infection associated with *Candida*, such as immunosuppression, antibiotics, age, smoking and dentures⁽³⁾.

Candida infections are very common amongst denture wearers and this could be related to the reason that wearing dental prosthesis is considered as a predisposing factor for colonization of the mouth with *Candida* species⁽⁴⁾. A study by Pires et al.⁽⁵⁾ revealed that the prevalence of *Candida* ranged from 60-100% in denture wearers. The rationale behind the increased prevalence of *Candida* in denture wearers could be that the transient microenvironment underneath the denture favors retention of food debris and consequently microorganisms^(5,6). Moreover, acrylic resin dentures can act as a plaque retentive factor and further enhance colonization of *Candida*⁽⁶⁾.

Age is another factor linked to *Candida* infection, since being elderly is usually associated with systemic disease, decreased manual dexterity, change in quantity and quality of saliva and thus changes in the oral environment that enhance the growth of *Candida*⁽⁷⁾. Furthermore, Soysa and Ellepola⁽⁸⁾ demonstrated that cigarette smoking increases overgrowth of *Candida* via its effect on saliva and oral commensals, mainly *Candida*.

The increase in the number of *Candida* is the first step toward the infection process and thus causing different degrees of denture stomatitis of mucosa in contact with the fitting surface of the denture^(6,9). Prevalence of *Candida*-associated denture stomatitis has been reported in 60-65% of individuals with a dental prosthesis⁽¹⁰⁾, with clinical features of edema and chronic erythema⁽⁸⁾. Although denture stomatitis mainly remains superficial and harmless, it might cause bleeding of oral mucosa and coexist with other conditions such as angular cheilitis and burning sensation beneath the denture⁽¹¹⁾, taste disorder⁽¹²⁾ endodontic infection⁽¹³⁾ as well as more serious conditions such as oral cancer⁽¹⁴⁾.

With population growth, in aging societies, there is more likelihood of people losing their teeth and having them replaced with a dental prosthesis⁽¹⁵⁾. Consequently, the chances of *Candida*-associated denture stomatitis rise. Denture stomatitis caused by a fungal infection in the human oral cavity⁽¹⁶⁾ and fungi can be detected on teeth surfaces, tongue, cheeks, oral mucosa, dental prosthesis and restorative material⁽¹⁷⁾. However, rough surface dental material harbors a higher number of yeasts^(18,19), which could be explained by the fact that rough surfaces act as a retentive factor and protect microorganisms from cleaning actions⁽²⁰⁾.

Several modalities have been proposed to provide successful management of the patient with denture stomatitis, such as correction of the nutritional deficiency, advice on cessation of any smoking habit, antifungal therapy and steroids. However, appropriate oral hygiene practice remains the main essential factor to remove *Candida* biofilms on host tissues and dental prostheses⁽⁴⁾.

This study is directed toward investigating the effect of brushing frequencies, age and smoking habit on the levels of *Candida* in the saliva of subjects with removable partial or complete dentures.

Patients and methods

Study groups

Subjects wearing removable partial or complete dentures were recruited for this study. The study protocol was approved by the ethical committee of University Sains Malaysia. Recruitment was conducted among subjects attending the outpatient clinic from January to March 2011 in the School of Dental Sciences at University Sains Malaysia. Potential subjects were screened by a clinical investigator and evaluated for exclusion criteria before being invited to take part in the study. The exclusion criteria were patients with systemic disease, active oral disease and history of antibiotic in the last three months. After obtaining written consent, the age, sex, frequency of brushing (regardless of any method or materials used for brushing), type of denture (denture's age did not take into account) and smoking habit were recorded, followed by salivary sample collection.

Sample collection and Candida identification

Salivary samples were collected by oral rinse technique. The participants instructed to rinse their mouth thoroughly with sterile phosphate buffer saline (10 ml) for 60s and the sample collected in a sterile container, stored in a cool box and then transported to the oral microbiology laboratory for processing. The oral rinse sample centrifuged at 1700g for 10 min then the supernatant discarded. One ml of concentrated saliva sample used for culturing. Serial decimal dilutions of samples were prepared in Phosphate-buffered saline. Aliquots of 0.1 ml were cultured in duplicate on Sabouraud Dextrose Agar (Himedia, India) and incubated at 37°C for 48 hrs⁽²¹⁾.



Figure 1: Candida growth on Sabouraud Dextrose Agar

The growth of Candida species was identified by smooth, white or creamy colored buttery colonies (Figure 1). Candida species were further identified by Gram stain as Gram-positive budding shaped yeast cells (Figure 2). The numbers of colonies were determined using the aCOLyte colony counter (Symbiosis, USA) (Figure 3 and 4). A pilot study on five samples was conducted to choose the appropriate dilution. The samples above or below the sensitivity (30- 300 CFU) of the machine were reanalyzed and the final numbers of CFU per mL (mean of duplicate samples) were obtained taking the dilution factor into account.

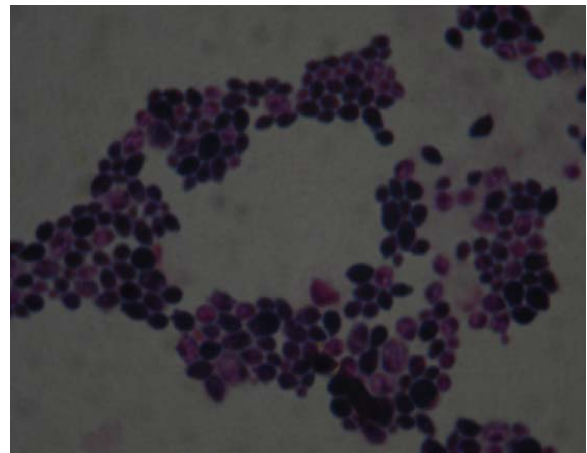


Figure 2: Gram stain showing Candida as gram-positive round cells



Figure 3: aCOLyte colony counter from symbiosis



Figure 4: aCOLyte colony counter connected to the computer.

Statistical analysis

The normality test (Shapiro-Wilk test) for continuous data was performed and the data were then subjected

to an appropriate test. The statistically significant difference between cleaning frequencies was conducted by Kruskal–Wallis test. The Mann-Whitney test was used to determine statistically

significant differences in CFU between partial and complete denture wearers and smoker versus non-smoker. Spearman's correlation was used to determine the correlations of CFU with age and brushing frequencies. Statistical significance was defined as $p \leq 0.05$ and all calculations were conducted using the SPSS software package (version 21; SPSS Inc., Chicago, IL, USA). The null hypothesis was that none of the above variables associated with CFU. Advice on data analysis was provided by an expert statistician.

Results

Ninety-nine subjects (38 male and 61 female) were recruited with the mean age of 59.9 ± 10 , ranging from 39 to 80 years (18%: 39-49 years, 32%: 50-59 years, 32%: 60-69 years, 18%: 70-80 years). Forty-five subjects were wearing complete dentures and 28 subjects were smokers. Thirty-seven subjects cleaned their denture once a day, 39 subjects twice a day and 23 subjects three times a day.

Candida species were isolated from 99% of the subjects. Analysis of saliva samples showed a statistically significant difference in CFU levels in partial denture wearers (median 1.5×10^6 mL⁻¹) compared to complete denture wearers (median 1.1×10^6 mL⁻¹) (Mann-Whitney test, $P = 0.01$) (Figure 5). Moreover, the Kruskal-Wallis test demonstrated statistically significant differences ($P < .001$) in CFU levels between subjects cleaning their denture once (median 2.3×10^6 mL⁻¹), twice (median 1.1×10^6 mL⁻¹) and three times a day (median 0.5×10^6 mL⁻¹) (Figure 6). There was no statistically significant difference in CFU levels between smoker (median 1.2×10^6 mL⁻¹) and non-smokers (median 1.3×10^6 mL⁻¹) individuals (Mann-Whitney test $P > 0.05$) (Figure 7).

Spearman's correlation also showed a statistically significant correlation between CFU levels and cleaning frequencies ($r = 0.8$, $P < .001$). There was no statistically significant correlation between CFU and age ($P = >0.05$).

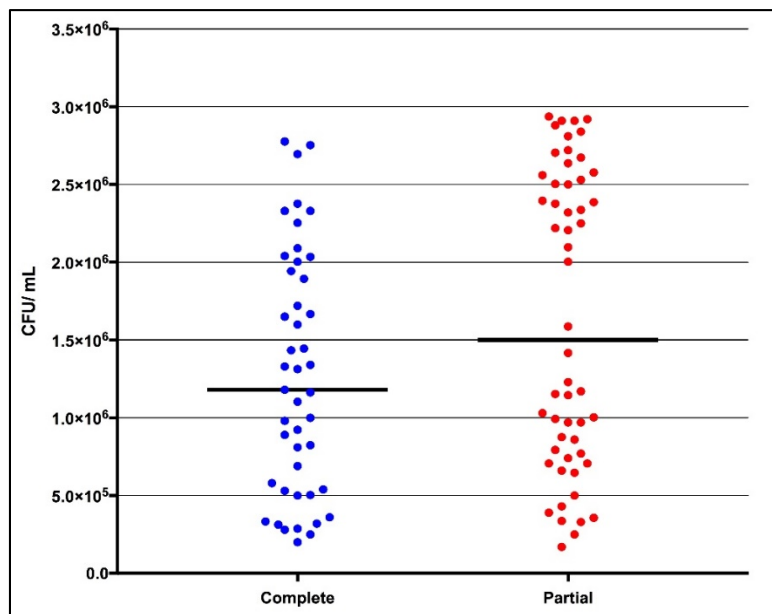


Figure 5: Comparison of median CFU between partial and complete denture wearers.

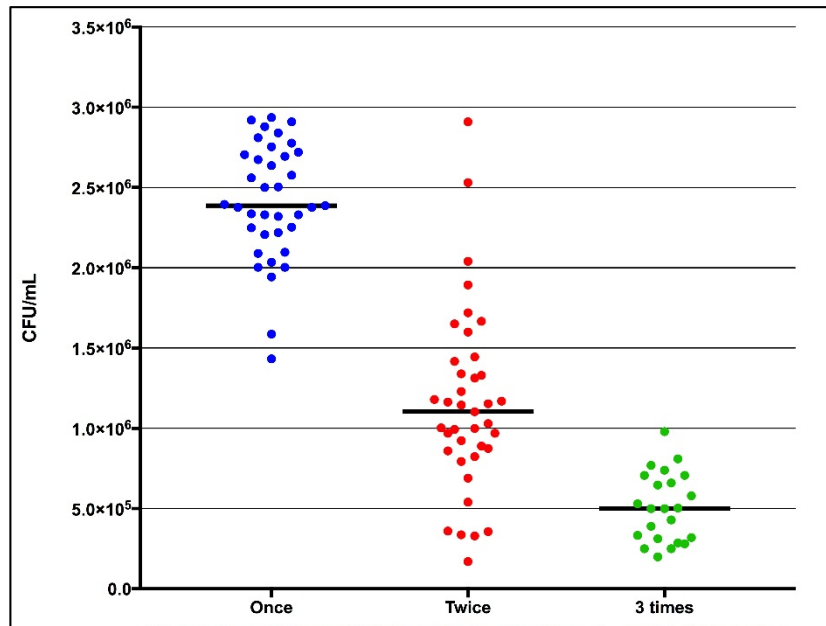


Figure 6: Comparison of median CFU in subjects with different denture brushing frequencies.

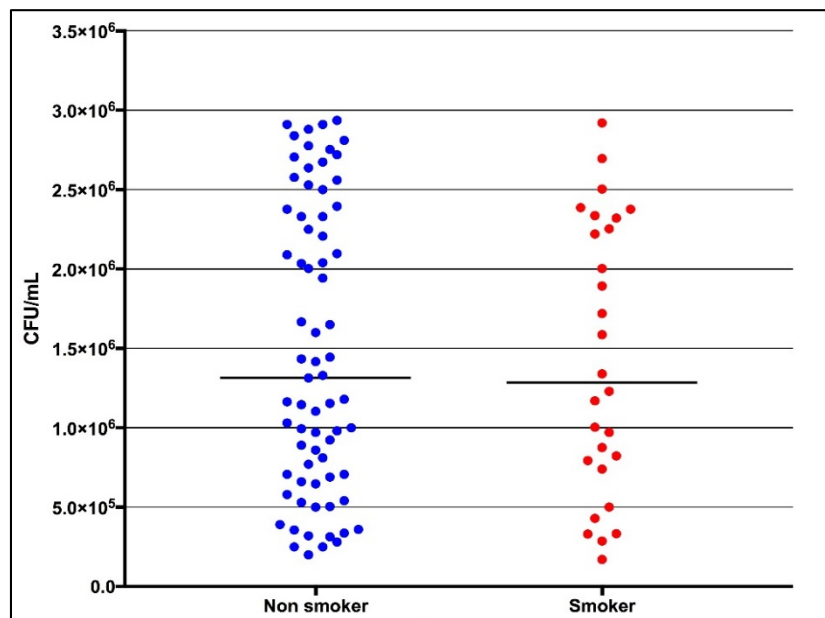


Figure 7: Comparison of median CFU between smoker and non-smoker subjects.

Discussion

This study found that the number of *Candida* species decreases significantly with increased cleaning frequencies and is statistically significantly higher in partial denture wearers than complete denture wearers. However, there were no statistically significant differences between smokers and non-smokers plus no correlation between CFU and age of subjects was detected. To our knowledge, this is the first time that the number of *Candida* species in the saliva of removable denture wearers has been investigated against frequencies of cleaning the denture.

As denture brushing frequencies increased, the numbers of *Candida* in the saliva decreased (Figure 6). Furthermore, cleaning frequencies were inversely proportional to the levels of *Candida* species in saliva ($r= 0.8$, $P= <.001$). Moreover, levels of *Candida* were demonstrated to be statistically significantly higher in partial denture wearers than complete denture wearers, which might be associated with the presence of teeth in partial denture wearers that provide further surfaces within the oral cavity that are difficult for patients to clean, a result that is in accordance with data published by Gusmão et al.⁽²²⁾.

There are conflicting data regarding the association between the number of *Candida* species in oral cavity and smoking habit. Studies by Abu-Elteen and Abu-Elteen⁽²³⁾ and Shin et al.⁽²⁴⁾ demonstrated higher levels of *Candida* species in oral cavity of smokers than that of non-smokers, which is not in agreement with the result of this study and this could be due to the fact that in this study the number of cigarettes per day and duration of smoking was not taken into account. However, our data were in accordance with other studies conducted by Masipa et al.⁽²⁵⁾ and Darwazeh et al.⁽²⁶⁾.

Again there are conflicting results regarding the relationship between a number of *Candida* and age of subjects. The data of this study did not show any correlation between age of patients and number of CFU, and this is not in line with Loster et al.⁽²⁷⁾ that showed a higher prevalence of *Candida* in complete denture wearers above 50 years old. The related points to consider are in this study both complete and partial denture wearers included and our sample size is not as large as of Loster et al.⁽²⁷⁾. Furthermore, 82% of subjects investigated in this study were

above 50 years old. Consequently, there were not enough samples below 50 years old to compare.

Conclusions

This study has demonstrated that subjects with higher cleaning frequencies have lower levels of *Candida* species and are thus less likely to develop denture stomatitis. Partial denture wearers need to clean their denture and remaining teeth more frequently, as higher levels of *Candida* were detected. Further studies on duration of cleaning, different cleaning techniques and cleaning materials need to be conducted to reveal the exact effect of brushing on the number of *Candida*. Furthermore, a longitudinal study needs to be carried out to show the recolonization time after denture brushing and the effect of brushing frequency on the reduction of denture stomatitis.

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