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The relationship between oral hygiene and oral colonization with *Candida* species in healthy adult subjects*

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Darwazeh AM-G, Hammad MM, Al-Jamaei AA. The relationship between oral hygiene and oral colonization with *Candida* species in healthy adult subjects.

Abstract: Poor oral hygiene has been frequently suggested as a predisposing factor for oral candidal colonization, but the convincing evidence is lacking. *Objective:* To assess and compare oral candidal colonization, both quantitatively and qualitatively, in groups of healthy dentate subjects with different levels of oral hygiene as determined by the plaque index (PI) and gingival index (GI) scores. *Methods:* The concentrated oral rinse technique was used to isolate *Candida* species from 149 healthy dentate subjects. *Candida* species were cultured on Sabouraud's dextrose agar plates and identified by germ-tube test and the automated Vitek® system biochemical yeast card. According to the PI and GI scores, subjects were divided into different groups of oral hygiene level. *Results: Candida* species were isolated from 86 (57.7%) subjects. The prevalence of candidal carriage increased significantly as a function of age ($P = 0.023$), but was comparable between males and females (58.7% and 56.7% respectively; $P = 0.87$). Oral candidal carriage rate and density were not affected by the levels of dental plaque or gingival condition. The prevalence of oral candididal carriage was significantly higher in the subjects who were not using dental floss compared with those who were using dental floss ($P = 0.032$). *Conclusion:* Oral hygiene status, as determined by the PI and the GI scores *per se*, does not affect oral candidal colonization in healthy dentate subjects.

Key words: *Candida;* dental plaque; gingival index; gingivitis; oral *Candida*; oral hygiene; plaque index

Introduction

Oral candidal colonization and candidosis have recently received increased attention by the biologists, hygienists or clinicians; particularly following the emergence of human immunodeficiency virus (HIV) infection (1) and the wide spread use of immunosuppressant and corticosteroid therapy (2). *Candida* species are opportunistic pathogens, which constitute part of the oral commensal flora in about 2–70% of healthy individuals, but is responsible for causing infection if the host immune barriers are breached either on the local or systemic level (3). In this regard, *Candida* adhesion and subsequent colonization of the mucosal surfaces are considered essential step in the process of development of candidosis (4).

Several local factors in the oral cavity have been shown to influence candidal growth, such as carbohydrate-rich diet (5), co-existence of certain bacterial species (6), xerostomia and wearing of dentures (7). However, little is known about the requirements for a healthy dentate individual to acquire *Candida* intra-orally, or the natural barriers against carriage and remains *Candida*-free. In denture-wearing subjects, the studies have shown that poor denture cleanliness and hygiene were associated with increased oral candidal colonization (8) and development of *Candida*-associated denture stomatitis (8, 9). Reviewing the dental literature revealed that 'poor oral hygiene' has been frequently claimed to be a local predisposing factor for increased oral candidal carriage and candidosis in dentate subjects (10–12), but the convincing scientific evidence is still lacking. As the studies that investigated the relationship of oral hygiene to oral candidial colonization were performed on elderly subjects with systemic diseases (13, 14) or wearing removable dentures (9) or on HIV-infected patients (15), the aim of this prospective study was to assess and compare the oral candidal colonization, both quantitatively and qualitatively, in groups of healthy adult dentate subjects with different levels of oral hygiene.

Study population and methodology

The study subjects were selected randomly from the subjects attending the Initial Treatment Unit in the Dental Teaching Center/Faculty of Dentistry, Jordan University of Science and Technology after they signed a formal consent form approved by the Institutional Review Board. Subjects who were apparently fit and healthy were included in the study. Subjects with known disease or condition predisposing to gingival or periodontal disease, such as diabetes mellitus, anaemia or xerostomia were excluded from the study. Subjects who used antibiotics or corticosteroids, antifungal agent or antiseptic mouth wash over the past 6 months or who had clinical signs suggestive of oral candidosis or other mucosal abnormalities, women who were pregnant or on oral contraceptives, edentulous subjects and those who use orthodontic appliances or removable dental prosthesis were also excluded.

Demographic data of each subject and the type and frequency of dental home care procedures and smoking habit were recorded in a special proforma. A subject was considered smoker if he/she smoked 10 cigarettes or more daily.

Oral hygiene assessment

The plaque index (PI) (16) and gingival index (GI) (17) were adopted as parameters for oral hygiene assessment. According to the PI score, the subject's plaque status was assigned as follows:

- Very good <0.1.
- Good = 0.1–0.9.
- Poor = 1.0–1.9.
- Very poor = 2.0–3.0.

According to the GI score, the subject's gingival health was assigned as follows:

- No inflammation <0.1.
- Mild inflammation = 0.1–1.0.
- Moderate inflammation = 1.1–2.0.
- Severe inflammation = 2.1–3.

Microbiological assessment

The concentrated oral rinse technique (18) was used for oral candidal sampling. The processed oral rinse samples were inoculated on plates of Sabouraud's dextrose agar (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated aerobically at 37°C for 48 h. *Candida* colonies were recognized according to their morphology and by observing the blastospore phase microscopically in direct wet film under ×40 objective. *Candida albicans* and other *Candida* species were identified by using the germ-tube test (19) and the AutoMicrobic Vitek® system (Vitek 60, Bio-Merieux, France) (20). The number of *Candida* colonies on each plate was quantified by the number of colony forming unit per 1 ml of the rinse (CFU ml^{-1}). All sampling and scoring was performed by the same investigator (AAA), between 9.30 and 11.30 a.m. at least 2 h after eating, drinking or oral hygiene practice.

Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS) version 11.0. The difference between means was tested by using Student's *t*-test or Mann–

Whitney test (for non-normally distributed data). The chi-squared test was used to analyse the difference between frequencies in groups. The relationship of oral candidal density to the plaque status or gingival status was tested using one-way ANOVA test. P-value < 0.05 was considered significant.

Results

The study included 149 healthy adult dentate subjects closely matched for gender and age, with age ranging between 18 and 48 years. The mean age of the study subjects was 25.3 (±7.6) years. The mean age for males was 24.9 (±6.9) and 25.8 (±8.2) years for females. The characteristics of the study subjects are described in Table 1. None of the subjects examined had severe gingival inflammation. The mean PI score was significantly higher among smokers compared with non-smokers (1.59 ± 0.46 versus 1.32 ± 0.48 respectively; $P = 0.005$) but not the mean GI score (1.28 ± 0.30 versus 1.37 ± 0.35 respectively; $P = 0.19$).

Candida species were isolated from 86/149 (57.7%) subjects and comparably from males [44/75 (58.7%)] and females [42/74 (56.7%); $P = 0.87$]. The different *Candida* species isolated are presented in Table 2. The prevalence of other yeast species (other than *Candida*) was as follows: *Saccharomyces cerevisiae* 6 (4%) subjects, *Aspergillus niger*, *Yarrowia lipolytica*, *Cryptococcus lauronitii* each in one subject (0.7%), but were not counted with candidal isolation.

When the subjects were divided into subgroups according to age with 5-year interval, one-way ANOVA test showed that the prevalence of candidal carriage increased significantly as a function of age ($P = 0.023$).

Table 1. **The characteristics of the study subjects**

Variable	Male (n = 75) n (%)	Female (n = 74) n (%)	Total (n = 149) n (%)
Cigarette smokers	31 (41.3)	4 (5.4)	35 (23.5)
Brushing teeth	74 (98.7)	74 (100)	148 (99.3)
Dental flossing	7 (9.3)	9 (12.2)	16 (10.7)

Table 2. ***Candida* species isolated from the oral cavity of the study subjects**

Candida species	Frequency of isolation n (%)
C. albicans	78 (52.4)
C. kefyer	9 (6.0)
C. glabrata	3 (2.0)
C. lambica	1 (0.7)
Total	91*

*More than one *Candida* species was isolated from five subjects.

The statistical analysis revealed no significant difference in either the prevalence of oral candidal carriage ($P = 0.94$) or the density of isolation ($P = 0.36$) between the subjects with good, poor or very poor plaque status. Similarly, there was no significant difference in either the prevalence of oral candidal carriage ($P = 0.85$) or the density of isolation ($P = 0.24$) between the subjects with mild and moderate gingival inflammation (Table 3).

The prevalence of oral candidal carriage was significantly higher ($P = 0.032$) in the subjects who were not using dental floss [81/133 (60.9%)] compared with those who claimed that they were regularly using dental floss [5/16 (31.2%)], but there was no significant difference in the mean CFU ml^{-1} between the two subgroups [888 (SD 1218) versus 722 (SD 502) respectively; $P = 0.9$]. No attempt was made to test oral candidal colonization in relation to tooth brushing as there was only one subject who reported not brushing the teeth.

The statistical analysis showed comparable prevalence of oral candidal carriage between smokers and non-smokers [21/35 (60%) versus 65/114 (57%) respectively; $P = 0.85$]. Nevertheless, the mean CFU ml^{-1} was significantly higher among smokers compared with non-smokers [1495 (SD 1586) versus 656 (SD 954) respectively; $P = 0.031$].

Discussion

It is believed that an individual's oral hygiene is directly related to the amount of plaque built up on teeth. Therefore, oral hygiene status was assessed in this study using Silness and Loe PI (16). Improper oral hygiene leads to accumulation of dental plaque and development of gingivitis, which justifies using the GI (17) as another parameter for oral hygiene assessment.

The higher PI among smokers has been reported previously (21) and was partly explained by personality traits leading to decreased attention to oral hygiene (22) and by the higher salivary calcium concentration (23). In addition, higher growth rate for oral *Streptococcus sanguis* and *S. mutans* has been observed in smokers, the microbes which believed to form the initial part of the dental plaque ecology (24).

Whether tobacco smoking increases oral *Candida* carriage (25) or not (26, 27) is still a matter of debate. Our results demonstrated comparable rates of candidal carriage between smokers and non-smokers. However, the higher candidal count observed among the smokers in our study may be attributed to the aromatic hydrocarbons in tobacco, which have been shown to act as nutrients to the yeast cells (28). In addition, smoking may indirectly increase the level of salivary glucose, which

Table 3. **The relationship of oral candidal colonization to oral hygiene status**

Variable	*Candida* carriage *n* (%)	*P*-value	Mean CFU ml^{-1} (SD)	*P*-value
Plaque status				
Good (*n* = 26)	15 (57.7)	0.9	919 (1053)	0.4
Poor (*n* = 95)	54 (56.8)		736 (1126)	
Very poor (*n* = 28)	17 (60.7)		1205 (1461)	
Gingival status				
Mild inflammation (*n* = 39)	22 (56.4)	0.8	1115 (1306)	0.2
Moderate inflammation (*n* = 110)	64 (58.2)		773 (1141)	

enhances yeast growth. Also, smoking can depress the activity of oral leucocytes and other non-specific immune defences (28).

The overall oral carriage rate of *Candida* species in this group of adult healthy dentate subjects was 57.7%. Other studies reported 52% (29), 53% (27) and 58.6% (30) among similar group subjects. The predominance of *C. albicans* isolation was expected as this species is known to be the most common *Candida* species isolated from human in health and disease (3). Interestingly, this study demonstrated a significant increase in the candidal carriage rate with increasing age. This could be explained by the physiological variations associated with age, such as body fluid alteration, change in the living and diet habits and alterations in the ecological environment of the oral cavity (3).

Several clinical studies have indirectly extrapolated the presence of a relationship between oral hygiene and candidal colonization among geriatric patients in long-term hospital care (31) or among residents in long-term care facility (9, 14, 32). In these studies, the group of subjects who received professional oral and denture health care by dental hygienist showed a marked reduction in the prevalence and count of *Candida* species compared with others who did not receive similar care. However, such patients are known being liable for increased oral *Candida* carriage and candiosis possibly because of the existing systemic diseases, medications and wearing of removable prosthesis (10). In contrast, our prospective study demonstrates lack of association between the dental plaque status and oral candidal colonization in healthy adult dentate subjects, which agrees with the results of another study in a comparable population (27). Reviewing the dental literature reveals that the studies, which reported a significant association between oral hygiene status and increased candidal carriage rate and density among dentate healthy subjects, were performed on subjects under treatment with orthodontic appliances (33, 34). It was hypothesized that the insertion of such appliances may induce ecological alterations and create a new micro-environment, which may favour candidal growth (33).

Candida species present in the dental plaque in the form of biofilm. A recent study (6) has investigated the effect of co-existence of common oral and dental plaque bacterial species including the putative periodontal pathogens *Provotella nigrescens* and *Porphyromonas gingivalis*, *Actinomyces israelii*, *Lactobacillus acidophilus*, *S. mutans* and *S. intermedius* on candidal growth in a model of dental plaque. This study reported an inhibitory effect of these organisms on candidal growth in the biofilm. This finding may partly explain why in some studies, *Candida* species were isolated from the oral cavity and saliva both quantitatively and qualitatively more than from the dental plaque (35) and subgingival samples (36). *Candida albicans* cells in biofilm are more resistant to antifungal agents such as amphotericin B, fluconazole, nystatin and chlorhexidine mouth wash than the planktonic cells (37). Therefore, one can speculate that some *Candida* cells in dental plaque might serve as a reservoir for recurrent episodes of infection in patients who received antifungal therapy for treatment of oral candidosis (38). Our finding of lower prevalence of oral *Candida* species among individuals who claimed using dental floss regularly compared with those who admitted not to floss their teeth should be interpreted with caution in view of the objective finding of lack of association between dental plaque and gingival inflammation status and oral candidal carriage.

In this study, none of the subjects was diagnosed as having severe gingivitis. One study reported a significant relationship between the presence of oral candidosis and linear gingival erythema in HIV-infected patients (15). We observed no significant relationship between gingival status and oral candidal carriage rate or density, which was the conclusion of other study among healthy adults visiting dental clinics (27). In clinical terms, according to the results of this study, plaque status or gingival status *per se* in healthy adults who do not wear removable dental prosthesis may not be key factors which promote oral yeast colonization and subsequent morbidity in the absence of other aetiological factor for oral candidosis. It is possible that subjects with altered defence mechanisms, either on the local or systemic level with lower standard of oral

hygiene, may become more susceptible to oral candidal colonization and infection compared with those with proper oral hygiene, which warrants further investigations. Hypothetically, *Candida* cells may preferentially colonize the dental plaque in subjects with poor oral hygiene who have higher level of dental plaque and gingival inflammation. This may explain partly the lack of relationship of oral hygiene and oral candidal colonization and warrants culturing samples of the dental plaque, along with oral rinse samples in future studies to test this hypothesis. However, putting these data together with that obtained from studies on medically compromised subjects (9, 13, 14, 31, 32) or those wearing dental prosthesis such as orthodontic appliances or dentures (33, 34) signifies the importance of maintaining high standards of oral and dental hygiene by the dental hygienists to prevent oral candidosis, especially in predisposed subjects.

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