

The Relationship between Tobacco Smoking and Oral Colonization with *Candida* Species

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Abstract

Aim: The aim of this study was to assess and compare the quantitative and qualitative oral colonization of *Candida* species between a group of healthy tobacco smokers and a comparable group of nonsmokers, and to investigate a possible correlation between oral candidal colonization and the quantity or duration of the smoking habit.

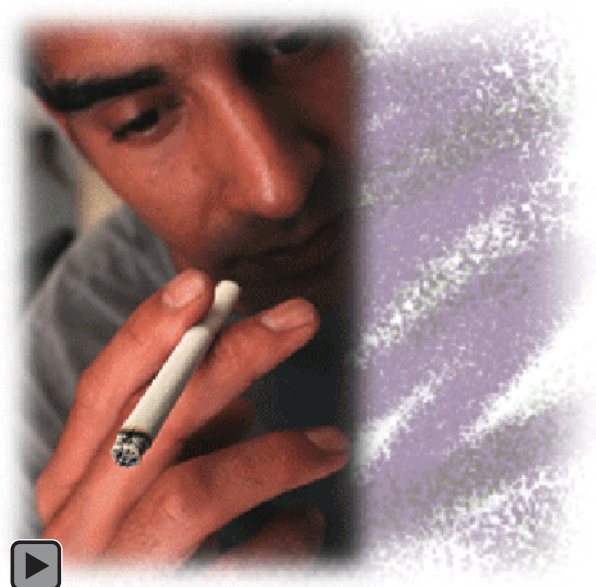
Methods and Materials: Fifty smokers and 50 nonsmokers were included in the study. *Candida* species were isolated using the concentrated oral rinse (COR) technique and identified using the germ tube test and API 20 C AUX yeast identification system.

Results: Overall candidal transmission was 84 percent. *Candida* species were isolated from 42 (84 percent) of the smokers and 37 (74 percent) of the nonsmokers ($p>0.05$). The mean CFU/ml were 333 (SD=358) and 268 (SD=332), respectively ($p>0.05$).

Conclusion: Tobacco smoking did not appear to increase oral colonization with *Candida* species in healthy subjects.

Clinical Significance: The effects of smoking on oral tissues and the mechanisms by which *Candida* proliferate intra-orally as a result of cigarette smoking warrant additional study.

Keywords: *Candida albicans*, *Candida* species, tobacco, epidemiology, smokers and nonsmokers.



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Introduction

Candida species constitute a part of the human oral commensal flora in 2 to 71 percent of healthy subjects.¹ Different environmental factors have been shown to increase asymptomatic oral candidal transmission such as wearing of removable dental prostheses,² salivary pH,³ and interaction between *Candida* species and

other commensal microflora.⁴ Whether tobacco smoking should be considered as one of these factors is still a matter of debate.

Several previous studies have reported that tobacco smoking, either alone or in combination with other systemic or local factors, is associated with increased oral candidal colonization or with the development of oral candidosis,⁵⁻⁸ while other studies have not shown this association.⁹⁻¹² Many of the previous studies in the field investigated the effect of tobacco smoking on oral *Candida albicans* colonization but ignored other species.^{5,9,10} The aim of this study was to assess and compare the quantitative and qualitative oral colonization of *Candida* species between a group of healthy tobacco smokers and a comparable group of nonsmokers, and to investigate a possible correlation between oral candidal colonization and the quantity or duration of the smoking habit.

Methods and Materials

The subjects in this study were randomly selected from a panel of individuals accompanying patients attending the Dental Teaching Center/Jordan University of Science & Technology for dental treatment. Because some studies have shown that subjects' gender may affect the prevalence of oral *Candida* due to the hormonal differences between males and females,^{10,11} only apparently healthy male dentate subjects, who were not wearing any removable dental prosthesis, were enrolled in the



study. Individuals who had more than four teeth extracted (excluding third molars) were excluded. Aiming to have both the smoker and nonsmoker groups matched for age, as much as possible, subjects who were below 18 years old or above the age of 50 years were not included in the study.

Each subject underwent a routine oral clinical examination. The subject's medical and dental history was reviewed. The average number of cigarettes smoked daily and the duration of the smoking habit, in years, were noted. Criteria for exclusion from the study were the following:

1. Subjects who had florid gingivitis, periodontitis, or prominent dental plaque deposition that could be detected visually and occluded the interdental space.
2. Subjects who used any medication known to predispose to oral candidosis, such as corticosteroids, antibiotics, or medication inducing xerostomia over the past six months.
3. Subjects who reported any systemic predisposing factor for oral candidosis, such as diabetes mellitus or anemia.
4. Subjects wearing removable dental prosthesis or orthodontic appliance.
5. Subjects who used antifungal agents or antiseptic mouthwash over the past six months.
6. Subjects with any oral mucosal abnormality suggestive for oral pathology such as leukoplakia, lichen planus, or any keratotic lesion or erythematous lesion.

A subject was classified as "smoker" who smoked at least 10 cigarettes per day for the last year. The "nonsmoker" was defined as someone who either never had smoked or who had quit smoking for at least one year prior to the study. Subjects who were using tobacco in any other forms also were not included in the study. All subjects signed an informed consent form approved by the research ethical committee at Jordan University of Science and Technology.

For oral candidal isolation, the concentrated oral rinse (COR) techniques as described by Samaranyake et al.¹³ was used. Each subject was supplied with 10 ml of sterile phosphate buffered saline (PBS, 0.1 M, pH 7.2) in a sterile universal container and asked to rinse his mouth

thoroughly for a full 60 seconds, after which time the rinse was expectorated into the container. The collected samples were either processed immediately or left on crushed ice until processed. The rinse container was then centrifuged at 2×10^3 g for 10 minutes, the supernatant was discarded, and the deposit was diluted with 1 ml PBS and vortex-mixed for 30 seconds for optimal microbial desegregation. Afterwards, a 0.5 ml sample of the mixture was inoculated on a Sabouraud's dextrose agar plate (Gibco, Paisley, Scotland). The number of yeast colonies on each plate was counted and multiplied by two to determine the number of colony-forming units per 1 ml of the rinse (CFU/ml). *C. albicans* and other *Candida* species were identified using the germ tube formation test in human serum¹⁴ and the yeast identification system API 20C AUX (bioMérieux, Marcy l'Étoile, France).¹⁵ All the clinical examinations and microbiological sampling were performed by one of the authors (AAA) between 9 and 11 a.m. None of the subjects had consumed any food or drinks, practiced any oral hygiene procedure, or smoked at least one hour before the sampling.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 11.0. The proportion of *Candida* carriers and noncarriers were compared by means of the chi-squared test. Because the numbers of CFU/ml were not randomly distributed, a Mann Whitney U test was used to compare quantitative candidal isolation between groups. A p-value <0.05 was considered statistically significant.

Results

The subjects of the study included 50 smokers and 50 nonsmoker male subjects matched for age. None of the subjects had clinical signs of oral candidosis as evidenced on clinical examination. The age distribution of the study subjects is

shown in Table 1. The mean number of cigarettes smoked in the smokers group was 21 cigarettes (SD=8.6) per day, with the range between 10 and 40 cigarettes and a median of 20 cigarettes. The mean duration of the smoking habit was 8 years (SD=5.5), with a range between 2 and 31 years with a median of 5.5 years.

Candida species were isolated from the oral cavity of 42 (84 percent) of the smokers and 37 (74 percent) of the nonsmokers without significant difference between the two groups ($p > 0.05$). Also the mean count of *Candida* colonies isolated from the smokers and nonsmokers was 333 CFU/ml (SD=358) and 268 CFU/ml (SD=332), respectively, with no statistically significant difference between the two groups ($p > 0.05$).

C. albicans was the most frequently isolated species from the subjects in both groups. Different *Candida* species isolated from the two groups and their frequency are presented in Table 2. *C. dubliniensis* was tentatively identified in one smoker and one nonsmoker subjects.

The smoker group was divided into subgroups according to the number of cigarettes smoked per day and the duration of smoking habit. Since each group did not have an equal number of smokers of matching age with the same frequency and duration of smoking habit, biostatistical advice was to correlate the CFU/ml with the number of cigarettes smoked per day and the duration of the smoking habit.

Correlation tests were performed to examine the correlation between subjects' age, the number of cigarettes smoked per day as reported by the subjects, and the duration of the smoking habit expressed in years, with the number of CFU/ml. There was no significant correlation (r) between the age and CFU/ml in the smokers ($p = 0.336$). However, a positive correlation was detected

Table 1. The age distribution of the study subjects.

Subjects	Mean Age (SD) (years)	Median Age (years)	Age Range (years)
Smokers (n=50)	24.48 (7.92)*	25	19-48
Nonsmokers (n=50)	33.58 (11.30)*	31.5	18-58

SD: Standard deviation from the mean
* $p > 0.05$ (Student's t test)

Table 2. Type and frequency of *Candida* species isolated using the concentrated oral rinse technique.

<i>Candida</i> species	Smokers (n=42) No. (%)	Nonsmokers (n=37) No. (%)
<i>C. albicans</i>	23 (54.8)	24 (64.9)
<i>C. famata</i>	8 (19)	4 (10.8)
<i>C. spherical</i>	4 (9.5)	2 (5.4)
<i>C. kefir</i>	5 (12)	1 (2.7)
<i>C. guilliermondi</i>	1 (2.3)	3 (8.1)
<i>C. dubliniensis</i> *	1 (2.3)	1 (2.7)
<i>C. parapsilosis</i>	0 (0.0)	1 (2.7)
<i>C. tropicalis</i>	0 (0.0)	1 (2.7)
Total	42 (100)	37 (100)

*Tentatively identified.

Table 3. Oral candidal frequency rate in different age groups.

Age Group (years)	Smokers No. (%)	Nonsmokers No. (%)
<20	1/1 (100)	1/1 (100)
20-29	27/33 (81.8)	17/21 (80.9)
30-39	7/9 (77.8)	9/13 (69.2)
40-49	7/7 (100)	10/15 (66.7)
Total	42/50 (84)	37/50 (74)

between the number of CFU/ml isolated and the age in the nonsmokers ($r=0.39$; $p=0.018$). The study subjects also were divided into subgroups according to age (Table 3). A one-way ANOVA test showed that the prevalence of oral candidal colonization was not significantly different among the various age groups either for smokers ($p=0.69$) or nonsmokers ($p=0.85$).

There was a marginally significant positive correlation between CFU/ml and the number of cigarettes smoked per day ($r=0.30$; $p=0.05$), but not with the duration of smoking habit ($p=0.53$). When the authors attempted to divide the smokers into subgroups according to the number of cigarettes smoked per day or the duration of their smoking habit to compare the prevalence of candidal colonization between the subgroups, statistical analysis was not feasible due to the small sample size in each subgroup.

Discussion

To study the possible effect of tobacco smoking on oral candidal colonization, it was essential to standardize the subjects under investigation and to eliminate, as much as possible, any factors known to predispose someone to oral candidal colonization except the one under investigation (smoking). Since oral candidal prevalence has been shown to be influenced by the wearing of removable dental prostheses² and gender,^{10,11} only fully dentate male subjects were included in this study.

The concentrated oral rinse technique used in this study is known for its superior sensitivity, both qualitatively and quantitatively, in the overall candidal sampling of the oral cavity.¹³ The overall oral candidal prevalence rate in this study is relatively higher than that generally reported in

the literature.¹ This could be related to the ethnic variations among different studies' subjects.¹ However, this also emphasizes the need for studies to assess oral candidal prevalence rate among a larger Jordanian population.

Although the relationship between cigarette/ tobacco smoking and oral candidal colonization has been investigated by a number of researchers, a consensus on this issue has not yet been reached. The differences in the sampling techniques and the population groups studied may partly explain the discrepancy in the results. Some studies have shown that the rate of oral candidal prevalence was higher in tobacco smokers than in nonsmokers,^{3,4,8} while others concluded the lack of a significant difference between the two groups.^{9-11,16,17} However, there is increasing evidence that tobacco smoking constitutes a significant risk factor for oral candidosis in diabetic patients,¹⁸ human immunodeficiency virus—infected patients,¹⁹ and cancer patients undergoing radiation therapy.²⁰ In addition, tobacco smoking appeared to increase the prevalence of erythematous candidosis among denture wearers²¹ and patients with candidal leukoplakia.²²

The data from this investigation are in agreement with other studies that reported that oral candidal colonization was comparable between tobacco smokers and nonsmokers.^{6,11,12,23} The studies that reported a remarkably higher prevalence of oral *Candida* among tobacco smokers may have included subjects who were susceptible to oral candidosis such as poorly controlled diabetic patients,¹⁸ patients undergoing radiation therapy,²⁴ HIV-infected patients,¹⁹ subjects who were wearing a removable dental prosthesis,⁵ or denture-wearing patients who were already complaining of oral candidal infections such as erythematous candidosis²¹ or candidal leukoplakia.^{10,22} When considering these reports altogether with the findings of this study, it is tempting to speculate that smoking, in combination with other factors, could enhance oral candidal growth and constitutes a significant risk for the development of oral candidosis. This hypothesis may be supported by our observation of the lack of oral candidosis among the study subjects (smokers and nonsmokers) despite the relatively high rate of oral candidal prevalence. The latter is possibly due to the strict study inclusion criteria where only dentate healthy subjects, free of known predisposing factors for oral candidosis or mucosal abnormality,

were included.

C. albicans was the most commonly isolated species in this study among both smokers and nonsmokers. This is consistent with others who reported that *C. albicans* is the most commonly isolated species from the oral cavity in health carriers²⁵ and oral candidosis.²⁶ *C. dubliniensis* was tentatively identified in one smoker and one nonsmoking healthy subject. The majority of *C. dubliniensis* reported in the literature have been recovered from the oral cavity, suggesting this species may be particularly adapted to oral colonization as a constituent of normal human oral flora, with a potential to cause clinical infection.²⁷ Although the majority of *C. dubliniensis* were isolated from HIV-infected patients, it was also isolated from HIV-negative subjects, including healthy persons.²⁸ Surprisingly, *C. glabrata*, though a common oral commensal, was not recovered from any of this study's subjects. However, the prevalence of different *Candida* species has been shown to vary between ethnic groups.¹

In view of the findings of the current investigation, additional studies are needed to examine the effect of tobacco smoking on oral candidal prevalence in standardized study populations. Other variables are recommended to be taken simultaneously into consideration in these studies such as salivary flow, saliva composition, and *Candida* adhesion to oral epithelial cells.

Conclusion

Within the confines of this study and based upon the evaluative criteria, the following conclusions can be drawn:

1. The prevalence and density of oral candidal colonization were both higher in the smokers compared to the nonsmokers; however, the difference was not statistically significant.
2. There was a marginally significant positive correlation between the number of cigarettes smoked per day and the density of candidal growth in oral rinse cultures.
3. It is recommended that a larger sample of subjects should be investigated in future studies to precisely clarify the relationship between tobacco smoking and oral candidal colonization.

Clinical Significance

The possible impact of smoking on oral tissues' immune mechanisms and the mechanisms by which *Candida* proliferate intra-orally as a result of cigarette smoking require more study to better understand the relationship between tobacco use and oral candidal colonization.

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