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

Azmi Mohammad-Ghaleb Darwazeh, BDS, MSc, PhD, FFDRCSI; Ziad Nawaf Al-Dwairi, BDS, PhD; Abd Al-Wahab Al-Zwairi, BDS, MDS

**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.

**Citation:** Darwazeh AM, Al-Dwairi ZN, Al-Zwairi AA. The Relationship between Tobacco Smoking and Oral Colonization with *Candida* Species. J Contemp Dent Pract [Internet]. 2010 May; 11(3):017-024. Available from: http://www.thejcdp.com/journal/view/volume11-issue3-al_dwairi

**Introduction**

*Candida* species constitute a part of the human oral commensal flora in 2 to 71 percent of healthy subjects. Different environmental factors have been shown to increase asymptomatic oral candidal transmission such as wearing of removable dental prostheses, salivary pH, and interaction between *Candida* species and...
other commensal microflora. 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, while other studies have not shown this association. Many of the previous studies in the field investigated the effect of tobacco smoking on oral Candida albicans colonization but ignored other species. 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, 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 eukoplakia, 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 Samaranayake et al. 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
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 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 \(2\times10^3\) 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 serum and the yeast identification system API 20C AUX (bioMérieux, Marcy l’Etoile, France). 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 age distribution of the study subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean Age (SD) (years)</th>
<th>Median Age (years)</th>
<th>Age Range (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers (n=50)</td>
<td>24.48 (7.92)*</td>
<td>25</td>
<td>19-48</td>
</tr>
<tr>
<td>Nonsmokers (n=50)</td>
<td>33.58 (11.30)*</td>
<td>31.5</td>
<td>18-58</td>
</tr>
</tbody>
</table>

SD: Standard deviation from the mean
* \(p<0.05\) (Student’s t test)

C. albicans and other 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 (\(p>0.05\)).

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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 prostheses and gender, only fully dentate male subjects were included in this study.

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 prostheses and gender, 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. The overall oral candidal prevalence rate in this study is relatively higher than that generally reported in

<table>
<thead>
<tr>
<th>Table 2. Type and frequency of <strong>Candida</strong> species isolated using the concentrated oral rinse technique.</th>
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<tbody>
<tr>
<td><strong>Candida</strong> species</td>
</tr>
<tr>
<td>C. albicans</td>
</tr>
<tr>
<td>C. famata</td>
</tr>
<tr>
<td>C. spherical</td>
</tr>
<tr>
<td>C. kefyr</td>
</tr>
<tr>
<td>C. guilliermondii</td>
</tr>
<tr>
<td>C. dubliniensis*</td>
</tr>
<tr>
<td>C. parapsilosis</td>
</tr>
<tr>
<td>C. tropicalis</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>*Tentatively identified.</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Table 3. Oral candidal frequency rate in different age groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Group</strong> (years)</td>
</tr>
<tr>
<td>&lt;20</td>
</tr>
<tr>
<td>20-29</td>
</tr>
<tr>
<td>30-39</td>
</tr>
<tr>
<td>40-49</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
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 carriers and oral candidosis. 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. Although the majority of C. dubliniensis were isolated from HIV-infected patients, it was also isolated from HIV-negative subjects, including healthy persons. 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. 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.

References


**About the Authors**

**Azmi Mohammad-Ghaleb Darwazeh, BDS, MSc, PhD, FFDRCSI (Corresponding Author)**

Dr. Darwazeh is a professor in the Department of Oral Medicine and Surgery at Jordan University of Science and Technology in Irbid, Jordan. He is also the chairman of the oral medicine specialty committee for the Jordanian Medical Council.

e-mail: darwazeh@just.edu.jo

**Ziad Nawaf Al-Dwairi, BDS, PhD**

Dr. Al-Dwairi is an assistant professor and chairman of the Department of Prosthodontics at Jordan University of Science and Technology in Irbid, Jordan. His research interests include techniques of DNA extraction and polymerase chain reaction (PCR) and genetic polymorphism and relationship to oral *Candida* infections.

**Abd Al-Wahab Al-Zwairi, BDS, MDS**

Dr. Al-Zwairi is a postgraduate student on the faculty of dentistry at Jordan University of Science and Technology in Irbid, Jordan.

e-mail: dr_zwiri@yahoo.com

**Acknowledgement**

This study was kindly supported by a grant from the Deanship of Scientific Research at Jordan University of Science and Technology (Grant number 56/2006).