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Comparison of oral *Candida* carriage in waterpipe smokers, cigarette smokers, and non-smokers

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Abstract: The aim of the present cohort study was to compare oral carriage of *Candida* in waterpipe smokers (WS), cigarette smokers (CS), and non-smokers (NS). A total of 141 individuals (46 WS, 45 CS, and 50 NS) were included. A questionnaire was used to gather demographic information and data on the daily frequency and duration of smoking habits, the reasons for smoking, and daily oral hygiene maintenance habits. Oral *Candida* (C.) samples were cultured and yeast species were identified using polymerase chain reaction. Unstimulated whole salivary flow rate (UWSFR) was also recorded. The numbers of missing teeth (MT) were counted and tongue lesions were clinically identified. *C. albicans* was the most prevalent yeast species isolated from all groups. Oral *C. albicans* carriage was higher among WS ($P < 0.05$) and CS ($P < 0.05$) than among NS. Oral *C. tropicalis* carriage, age, UWSFR and the number of MT were comparable among the groups. Oral *Candida* carriage was significantly higher among WS and CS than among NS. Therefore it appears that WS and CS are at an increased risk of developing oral candida infections.

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Introduction

Oral yeasts, mainly *Candida* (C.) *albicans*, are commensal oral microbes (1). Some studies have reported that the carriage rate of oral *Candida* species in healthy subjects ranges between 17% and 75% (2,3). A significant risk factor for increased oral *Candida* carriage is habitual tobacco usage (cigarette smoking and tobacco chewing) (4,5). One possible reason for this is that chemicals in tobacco, such as nicotine, nitrosodietheinal amine and aromatic hydrocarbons, act as sources of nutrition for *Candida* species, thus facilitating their growth (6). However, advanced age, female gender, steroid therapy, wearing of dentures and immunosuppression (as in patients with oral cancer, chronic hyperglycemia and acquired immune deficiency syndrome) have also been associated with an increased degree of oral candidal carriage (2,3,7-12).

The waterpipe (synonymous with goza, hookah, narghile, and shisha) is a form of smoking that involves the passage of charcoal-heated air through a perforated aluminium foil and across flavoured tobacco to become smoke, which bubbles through the water before being inhaled (13). The tobacco used in waterpipes contains 2-4% nicotine. In several Arab countries such as Bahrain, Egypt, Israel, Jordan, Kuwait, Lebanon, Qatar, Saudi Arabia, and the United Arab Emirates, waterpipe smoking is a cultural norm (14-21). However, waterpipe smoking

has also become popular in several western countries including Australia, Canada, the United Kingdom, and the United States (22-28). There is an erroneous belief that waterpipe smoking is less injurious to health than cigarettes (29); however, several studies (30-32) have reported that as well as causing tachycardia and hypertension, waterpipe smoking also impairs pulmonary function. Furthermore, it has been proposed that one session of waterpipe smoking is equivalent to smoking nearly 100 cigarettes (33).

Since nicotine is a major component of the tobacco used in waterpipes and cigarettes, it has been hypothesized that oral *Candida* carriage is higher in waterpipe smokers (WS) and cigarette smokers (CS) in comparison to non-smokers (NS). Therefore, the aim of the present study was to compare oral *Candida* carriage and species prevalence among WS, CS, and NS.

Materials and Methods

The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the College of Applied Medical Sciences, King Saud University, Saudi Arabia (FR-0377-2016). The study subjects signed a consent form and were allowed to resign from the research project at any point with no consequences.

Recruitment of study participants

Between March 2016 and December 2016, a single-blind cross-sectional study was performed at the College of Dentistry, King Saud University, Riyadh, Saudi Arabia. Male participants in all groups were well matched for age and race and were recruited from the Outpatient Department of the College of Dentistry, King Saud University.

Inclusion and exclusion criteria

Subjects who had smoked waterpipes and cigarettes at least once daily for at least 12 months were categorized as WS and CS, respectively. Subjects who reported having never consumed any type of tobacco product were categorized as NS. Users of smokeless tobacco products, medically compromised subjects (such as those with diabetes or those undergoing cancer therapy), wearers of partial and/or complete dentures and patients who had used steroids, non-steroidal anti-inflammatory drugs, antibiotics and/or antifungal drugs within the previous 90 days were excluded.

Questionnaire

Information about mean age, sex, duration of smoking, the daily frequency of waterpipe or cigarette use, the reason for smoking, and the daily frequency of brushing of teeth and tongue surfaces was collected.

Unstimulated whole saliva collection and determination of the whole salivary flow rate

The participants were requested to visit an oral healthcare centre during morning hours (between 7:30 a.m. and 8:30 a.m.) in a fasting state for collection of unstimulated whole saliva (UWS) samples. All samples were collected by a single trained and calibrated examiner (FV). The *kappa* score for intra-examiner reliability was 0.88. For collection of UWS samples, all participants were comfortably seated and asked to allow saliva to accumulate in the mouth for 5 min. The participants were requested to abstain from swallowing and moving their cheeks, lips and tongue during this time. The participants then spat the UWS into a measuring cylinder. The unstimulated whole salivary flow rate (UWSFR) was determined and recorded in millilitres per minute (mL/min).

Collection and identification of oral *Candida* samples

The dorsal surfaces of the tongue and bilateral buccal mucosae were scraped with a cotton swab (Biomerieux S.A., Montalieu-Vercieu, France). The swab was then returned to a containment tube. *Candida* strains were cultured at 37°C in Sabouraud's dextrose agar (Becton, Dickinson and Company, Sparks, MD, USA). The cultures were inspected every 24 h until 1 week of incubation. Polymerase chain reaction (PCR) and DNA sequencing were used to identify the *Candida* species. Yeast cells were suspended in 200 µL of PCR-grade water for DNA isolation, and then a DNA preparation robot (MagNA pure, Roche Diagnostics GmbH, Mannheim, Germany) was used to obtain genomic DNA (34). In order to sequence the DNA and to conduct PCR analysis, a region (approximately 500 bp) of the 18S ribosomal ribonucleic acid gene was amplified using universal primers and DNA polymerase. A PCR purification kit (250 QIAquick Qiagen, GmbH, Hilden, Germany) was used to remove nucleotides from the PCR products. The purified PCR was then processed for DNA sequencing using capillary electrophoresis technology (ABI 310 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). To minimize the possibility of error during sequencing, strands of PCR-amplified DNA fragments were sequenced (35). For yeast identification, DNA sequences were assessed and examined using the BLAST-DNA database (36).

Lesions on the tongue and numbers of missing teeth

Standardized criteria were used to assess tongue lesions such as coated tongue (CT), fissured tongue (FT), hairy tongue

Table 1 General characteristics of the study groups

Parameters	Waterpipe smokers	Cigarette smokers	Non-smokers
n	46	45	50
Mean age	38.6 ± 3.2	40.2 ± 2.6	36.5 ± 1.7
Sex (Male)	46	45	50
Duration of smoking habit (in years)	5.6 ± 1.2	20.3 ± 5.4	–
Daily frequency of smoking (no. of times)	4.2 ± 0.3 times/day	20.3 ± 5.4 times/day	–
Duration of each smoking session	30.7 ± 6.5	6.2 ± 1.5	–
Daily oral hygiene maintenance			
Once daily (%)	84.7%	88.8%	86%
Twice daily (%)	15.3%	11.2%	14%
Tongue brushing			
Yes (%)	–	–	–
No (%)	–	–	–

Table 2 Oral candida species isolated from waterpipe smokers, cigarette smokers, and non-smokers

	Waterpipe smokers (*n* = 46)	Cigarette smokers (*n* = 45)	Non-smokers (*n* = 50)
	n (%)	*n* (%)	*n* (%)
Candida albicans	33 (71.7%)*	31 (68.9%)*	19 (38%)
Candida tropicalis	10 (21.7%)	11 (24.4%)	8 (16%)
Candida albicans + *Candida tropicalis*	2 (4.3%)	1 (2.2%)	3 (6%)
Candida parapsilosis	1 (2.3%)	2 (4.5%)	–
Candida krusei	–	–	–
Candida luscitanie	–	–	–
Candida glabrata	–	–	–
Candida gullerimondi	–	–	–
No candida species isolated	–	–	20 (40%)

*Compared with non-smokers ($P < 0.05$)

(HT), geographic tongue (GT), and median rhomboid glossitis (MRG) (37-41). The numbers of missing teeth (MT) were also recorded.

Statistical analysis

Data were assessed using the SPSS software package (Version 18, SPSS, Chicago, IL, USA). Comparisons between WS, CS and NS were performed using one-way analysis of variance. For multiple comparisons, Bonferroni post hoc test was used. The study sample size was also calculated (nQuery advisor, Statistical Solutions, version 7.0, Saugus, MA, USA). With the inclusion of 45 subjects per group, a study power was 90% (assuming a standard deviation of 1%) with a two-sided significance level of 0.05.

Results

Characteristics of the study cohort

One hundred and forty-one male subjects (46 WS, 45 CS, and 50 NS) were included. The mean ages of the WS, CS and NS were 38.6 ± 3.2, 40.2 ± 2.6, and 36.5 ± 1.7 years, respectively. The mean duration of tobacco smoking among CS was 20.3 ± 5.4 years. The mean daily frequency of smoking among CS was 20.3 ± 5.4 times daily, and the duration of each smoking session was 6.2 ± 1.5 min. Tooth brushing once daily was reported by 84.7% of WS, 88.8% of CS, and 86% of NS. None of the participants in any group reported brushing the dorsal surface of the tongue (Table 1). All WS reported that they had decided to smoke a waterpipe as a substitute after quitting cigarette smoking. All the CS reported that they were smoking cigarettes because it helped them alleviate psychological stress.

Unstimulated whole salivary flow rate

The UWSFR for WS, CS, and NS was 0.55 ± 0.2 mL/min, 0.54 ± 0.3 mL/min, and 0.55 ± 0.5 mL/min, respectively.

Oral *Candida* carriage

The most prevalent yeast species isolated from the oral cavity of WS, CS, and NS was *C. albicans* followed by *C. tropicalis*. Oral *C. albicans* carriage was significantly higher among WS (71.7%) ($P < 0.05$) and CS (68.9%) ($P < 0.05$) than among NS (38%). Oral *C. tropicalis* carriage among WS (21.7%), CS (24.4%), and NS (16%) was similar. *C. parapsilosis* was isolated from 2.3% of WS and 4.5% of CS. From 40% of the NS, no *Candida* species were isolated (Table 2).

Lesions on the tongue and numbers of missing teeth
None of the WS, CS, and NS demonstrated lesions on the tongue upon clinical oral examination. There was no significant difference in the numbers of missing teeth among WS (mean 5.2 teeth; range 4-8 teeth), CS (mean 5.6 teeth; range 4-8) and NS (mean 4.7 teeth; range 3-6 teeth).

Discussion

As far as can be ascertained from the indexed literature, this is the first study to have compared oral *Candida* carriage and species prevalence among WS, CS, and NS. We hypothesized that a) oral candida carriage and species prevalence would be significantly higher among WS and CS than among NS; and b) there would be no significant difference in oral *Candida* carriage and species prevalence among WS and CS. The present results support these hypotheses. An interesting finding of this study was that although the daily frequency of tobacco smoking was nearly 5 times higher in CS than in WS (who smoked a waterpipe approximately 4 times daily), oral *Candida* carriage remained comparable among the two groups. Various explanations for this can be proposed. Firstly, it is noteworthy that all WS (who had never smoked cigarettes) were using a waterpipe as a substitute for cigarettes, which also contain tobacco. Secondly, the overall daily duration of exposure to tobacco smoke in WS (approximately 128 min) and CS (approximately 125 min) was also roughly equivalent. It is therefore postulated that since all WS were former CS, and that overall daily exposure to tobacco smoke was similar between WS and CS, oral *Candida* carriage in the two groups was also similar. In an attempt to precisely assess oral *Candida* carriage in WS, it is essential to conduct further studies with a stricter definition of WS, that is, individuals who exclusively smoke a waterpipe and have never consumed tobacco in any other form.

Our results showed that the most prevalent oral yeast species isolated from all groups was *C. albicans*, thus supporting previous studies showing that *C. albicans* is an integral component of the normal oral flora (11,42). *C. albicans* is also the most common yeast species associated with the etiology of oral candidiasis (43). A perturbing finding of the present study was the isolation of *C. albicans* and *C. parapsilosis* from the oral cavity of 33 (71.7%) and 1 (2.3%) WS and 31 (68.9%) and 2 (4.5%) CS, respectively. *C. parapsilosis* has been highlighted as an emerging human pathogen and is a leading cause of invasive candidal disease (44). Patients in intensive care units, particularly those with fixed prosthetic devices and indwelling catheters, are susceptible to infection with *C. parapsilosis* (44). This suggests that tobacco smokers may exhibit multispecies yeast colonization, perhaps disposing them to a greater risk of oral candida infection.

Despite the fact that none of the WS, CS, or NS reported brushing the dorsal surface of the tongue during routine tooth brushing, visual examination revealed no clinical evidence of tongue lesions (such as MRG, CT, GT, HT, and/or FT) in any group. This may have been related to the systemic health status and numbers of MT in the study groups. Female gender, medical conditions such as chronic hyperglycemia, hyposalivation, an increased number of MT, cancer therapy (such as radiotherapy and/or chemotherapy) and advanced age are known to be significant risk factors for increased oral *Candida* carriage (45-47). In this regard, possible limitations of the present study may have been that the WS, CS, and NS we investigated were male, relatively young (approximately 40 years old) and showed no significant differences in UWSFR and the numbers of MT. Moreover, all the participants reported themselves to be systemically healthy. These limitations may also partly explain the absence of oral lesions in our patient population. It is tempting to hypothesize that a) oral *Candida* carriage is significantly higher in elderly (>70 years old) edentulous immunocompromised WS and CS than in younger dentate WS and CS, and that b) elderly immunocompromised WS and CS may also exhibit more oral lesions (such as MRG, CT, GT, HT, and/or FT) than systemically healthy NS. Further studies are warranted to confirm this.

There is a general misconception that waterpipe smoking is not as hazardous as cigarette smoking because tobacco smoke in a waterpipe is filtered through water, which absorbs most of the nicotine. Javed et al. (13) showed that periodontal inflammatory conditions were worse in WS and CS than in NS, although there was no significant difference in the severity of periodontal inflammation among WS and CS. The present authors support the results of Javed et al. (13), since oral *Candida* carriage was also comparable between WS and CS with no significant difference between the two groups. Moreover, according to the World Health Organisation Study Group on Tobacco Product Regulation, a single session of waterpipe smoking is equivalent to inhaling smoke from approximately 100 cigarettes (33). It is therefore highly recommended that community health awareness programs should routinely be conducted to educate the public about the detrimental effects of smoking (including waterpipes and cigarettes) on health, and the fact that WS and CS are as equally hazardous to health. “Anti-tobacco” and “quitting smoking” programs might

be useful strategies to help WS and CS quit their habits, which may in turn also help improve their overall quality of life.

Oral *Candida* carriage is significantly more frequent among WS and CS than among NS. Both WS and CS are at an increased risk of developing oral *Candida* infections.

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Conflict of interest

The authors have no conflict of interest to declare.

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