Oral *Candida* Carriage and Species Prevalence among Tobacco-Smokers and Non-Smokers with and without Type 2 Diabetic Mellitus

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Abstract

The aim of the present study was to assess the oral *Candida* carriage and species prevalence among tobacco smokers and non-smokers with and without type 2 diabetes mellitus (T2DM). Thirty-three type 2 diabetic smokers, 30 non-smokers with T2DM, 32 systemically healthy smokers and 30 non-smoking systemically healthy individuals were included. Information regarding age, gender, duration of T2DM, duration of smoking, daily frequency of smoking and daily oral hygiene maintenance protocols was collected using a questionnaire. In all groups, FBG levels were measured using a digital glucometer, unstimulated whole salivary flow rate was measured and oral yeast samples were collected by scraping the dorsum of the tongue with a sterile cotton swab. Oral yeasts were identified using culture method and DNA sequencing. *Candida* species were isolated from all smokers and non-smokers with T2DM. *Candida* species were isolated from 100% non-diabetic smokers 56.7% non-diabetic non-smokers. *Candida albicans* (*C. albicans*) was the most commonly isolated yeast species in all groups. *C. albicans* carriage was significantly higher in non-diabetic smokers as compared with non-smokers. There was no significant difference in oral *Candida* carriage in type 2 diabetic smokers and non-smokers. In patients with T2DM, oral *Candida* carriage and species prevalence is governed by hyperglycemia and the role of tobacco smoking in this regard seems to be rather secondary.

Key Words: Oral Candida, Type 2 diabetes, Smoking, Hyperglycemia

Introduction

Candida albicans (*C. albicans*) is the most common fungal species that is isolated from the oral cavity of healthy individuals [1-3]. It has been estimated that oral *Candida* species harbor the oral cavity of 17% to 75% systemically healthy individuals [4]. However, under opportunistic conditions, these fungi may become opportunistic pathogens [3]. Local risk factors have been associated with an increased oral *Candida* prevalence and carriage includes xerostomia, tobacco smoking, denture wearing and poor oral hygiene maintenance [5-8]. Systemic diseases such as poorly-controlled diabetes mellitus, acquired immune deficiency syndrome and renal disorders have also been associated with an increased oral *Candida* carriage, which make immunosuppressed patients more susceptible to develop oral candidiasis as compared with their systemically healthy counterparts [3,9-11].

Studies [3,12,13] have reported that oral *Candida* carriage is significantly higher in patients with chronic hyperglycemia (such as those with poorly-controlled diabetes) than non-diabetic controls. These results may be explained by the fact that a dry oral environment (due to xerostomia in patients with poorly-controlled diabetes) facilitates *Candida* stagnation and growth on oral surfaces, most commonly the dorsal surface of tongue [3,14]. Moreover an immunocompromised state in patients with chronic hyperglycemia may also facilitate oral *Candida* growth and proliferation.

The precise mechanism through which oral *Candida* carriage is affected by tobacco remains unclear. However, it has also been hypothesized the aromatic hydrocarbons (such as N-nitrosobenzylmethylamine) in tobacco smoke act as nutrients for Candida species thereby augmenting their growth [15]. Moreover, it has also been reported that *C. albicans* catalyzes the formation of N-nitrosobenzylmethylamine, which is an explanation for the high prevalence of *Candida*-associated leukoplakia among tobacco smokers as compared with non-smokers [16].

Since chronic hyperglycemia and tobacco smoking are independent risk factors for increased oral *Candida* carriage; it is hypothesized that oral *Candida* carriage and species prevalence is significantly higher in type 2 diabetic and non-diabetic smokers as compared to non-smokers with and without type 2 diabetes mellitus (T2DM). The aim of the present study was to assess the oral *Candida* carriage and species prevalence among tobacco smokers and non-smokers with and without T2DM.

Materials and Methods

Ethical issues

The present study was approved by the Research Ethics Review committee at the College of Dentistry Research Center, King Saud University, Riyadh, Saudi Arabia. Participation was voluntary and all participants signed a consent form before being included in the present investigation.

Inclusion and exclusion criteria

Patients with medically-diagnosed T2DM (fasting blood glucose [FBG] levels >125 mg/dl) were included [17]. Patients with self-reported systemic disease such as type-1 diabetes mellitus and prediabetes, HIV infection/acquired immune deficiency syndrome, cardiovascular disorders, epilepsy, hepatic disorders and renal disorders were not sought. In addition, pregnant patients, patients with a recent history of antibiotic and/or steroid intake, patients with overlapping teeth and denture wearers were also excluded.

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Study participants

Thirty-three type 2 diabetic smokers, 30 non-smokers with T2DM, 32 systemically healthy smokers and 30 non-smoking systemically healthy individuals were included. These individuals were recruited from a local residential area of Riyadh, Saudi Arabia and were examined at an oral healthcare center in Riyadh, Saudi Arabia. In all patients with T2DM, diabetes had been diagnosed in in accordance with the criteria proposed by the American Diabetes Association [17].

Questionnaire

A trained interviewer (FAA) gathered information regarding age, gender, education status (graduate level), duration of T2DM (in years), duration of smoking habit (in years), daily frequency of smoking and daily oral tooth brushing protocols (once daily, twice daily, three times or more or sometimes) from type 2 diabetic smokers and non-smokers and controls. Data regarding self-reported tongue brushing was also asked for. The patients were inquired whether or not they brushed the dorsum of their tongue once daily, twice daily, three times or more or sometimes.

Measurement of fasting blood glucose levels

In all groups, FBG levels were measured using a digital glucometer (Accu-Chek Activ, Roche Diagnostics, Mannheim, Germany) using the finger-prick method. Commercially available glucometer strips (placed in the g lucometer) were gently touched onto the blood drop and analyzed using a glucometer. After 15 seconds, the reading was obtained and recorded.

Collection of oral yeast samples

Participants were instructed to refrain from eating and drinking at least two hours before collection of yeast samples. Sampling was performed between 8:00 am and 1:00 pm. Oral yeasts are pre-dominant on the dorsal surface of tongue; therefore tongue surface scraping is a reliable method for detecting *Candida* species [18]. Each yeast sample was collected by scraping the dorsum of the tongue with a sterile cotton swab (COPAN, Amies Charcoal single swab, CE 0124, Italy). The swabs were returned to the containment tube immediately after sampling.

Identification of oral yeast samples

Identification to species level was determined by a yeast identification system (API 32-C System bioMériux yeast identification programme, Lyon, France). If identification was not possible with the API 32 system, the yeast isolate was subjected to molecular identification. For DNA isolation, yeast cells were suspended in 200µl sterile Polymerase Chain Reaction (PCR)-grade water and genomic DNA was prepared using MagNA pure (Roche Diagnostics GmbH, Mannheim, Germany) a DNA preparation robot [19]. For DNA sequencing and PCR analysis, a region (about 500-bp) of 18S ribosomal ribonucleic acid gene was amplified by PCR using universal primers and ampliTaq Gold DNA polymarase. Primers and free nucleotides from the PCR products were then removed by using QIAquick PCR purification kit (250) (Qiagen, GmbH, Hilden, Germany). The purified PCR products were processed for DNA sequencing by BigDye Terminator Cycle Sequencing using capillary electrophoresis technology in ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Both strands of PCR amplified DNA fragments were sequenced to avoid error of sequencing [20]. The DNA sequence was analysed by a software and searched in the Blast DNA database for yeast identification and typing [21].

Unstimulated whole salivary flow rate

The Unstimulated Whole Saliva (UWS) samples were collected as described elsewhere [22]. Patients were seated on a chair in a quiet room with the head slightly bent forward. Saliva was allowed to accumulate in the patients' mouth (without any stimulation) for 5 minutes following which they were instructed to expectorate into a funnel connected to a gauged measuring cylinder. The patients were instructed to refrain from swallowing and moving the lips and tongue during saliva collection. Unstimulated Whole Salivary Flow Rate (UWSFR) was measured and recorded in milliliters per minute (ml/min). Immediately after collection, UWS samples were immediately transferred to disposable eppendorf tubes and placed on ice. UWS samples were aliquoted and frozen at -80^{o} Celsius. All UWS samples analyzed within 6 months of collection.

Statistical analysis

Statistical analysis was performed using a software program (SPSS, Version 18, Chicago, IL. USA). With inclusion of at least 30 individuals per group (assuming a standard deviation of 1.0%) the study power was estimated to be 85% at a two-sided significance level of 0.05. Intergroup comparisons were performed using one-way analysis of variance and for multiple comparisons Bonferroni *post hoc* test was used.

Results

Characteristics of the study population

The mean ages of smokers and non-smokers with and without T2DM were comparable and all participants were male. UWSFR was significantly higher in non-diabetic smokers ($P<0.05$) and non-smokers ($P<0.05$) as compared to smokers and non-smokers with TDM. UWSFR was comparable among type 2 diabetic smokers and non-smokers and non-diabetic smokers and non-smokers. FBG levels were significantly higher in smokers ($P<0.05$) and non-smokers ($P<0.05$) with T2DM as compared with non-diabetic smokers and non-smokers (*Table 1*). There was no significant difference in FBG levels among smokers and non-smokers with T2DM and non-diabetic smokers and non-smokers (*Figure 1*). All smokers and non-smokers with T2DM reported to brush their teeth once daily. Fifty percent of non-diabetic non-smokers and 68.7% non-diabetic smokers reported to brush their teeth one daily. Non-diabetic non-smokers (16.7%) reported to sometimes brush their dorsum of tongue (*Table 1*).

Oral *Candida* carriage and species prevalence

Candida species were isolated from 100% non-diabetic smokers 56.7% non-diabetic non-smokers. *C. albicans* was the most commonly isolated yeast species among patients with T2DM and controls. *C. albicans* carriage was significantly higher in type 2 diabetic smokers as compared with non-diabetic smokers and non-diabetic non-smokers ($P<0.05$). There was no significant difference in oral *C. albicans* carriage among smokers and non-smokers with T2DM. Among patients without T2DM, *C. albicans* carriage was significantly higher in smokers as compared with non-smokers ($P<0.05$) (*Table 2*). *C. tropicalis* was the second most commonly isolated oral *Candida* species, which was

Table 1. *Characteristics of the study population.*

Parameters	**Type 2 diabetic smokers** (*n*=33)	**Type 2 diabetic non-smokers** (*n*=30)	**Non-diabetic smokers** (*n*=32)	**Non-diabetic non-smokers** (*n*=30)
Mean age in years (range)	50.6 ± 6.6	51.4 ± 6.2	50.5 ± 6.4	52.6
Mean duration of smoking(in years)	28.5 ± 6.2	—	27.6 ± 8.5	—
Mean number of cigarettes smoked daily	14.4 ± 8.6	—	20.1 ± 3.1	—
Unstimulated whole salivary flow rate (mg/dl)	0.2 ± 0.1*†	0.2 ± 0.1	0.5 ± 0.4*	0.5 ± 0.3†
Oral hygiene maintenance				
Once daily (%)	100	100	68.7	50
Twice daily (%)	—	—	31.3	50
Tongue brushing				
Sometimes (%)	—	—	—	16.7

* P<0.05 † P<0.05 ‡ P<0.05 § P<0.05

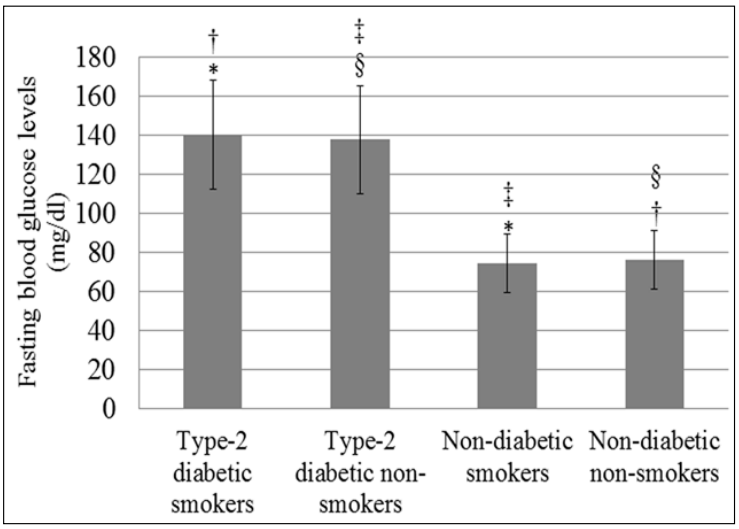

Figure 1*. Difference in FBG levels among smokers and non-smokers with T2DM and non-diabetic smokers and non-smokers.*

Table 2. *Oral Candida species isolated from smokers and non-smokers with and without type 2 diabetes.*

Oral *Candida* species	**Type 2 diabetic smokers** (*n*=33)	**Type 2 diabetic non-smokers** (*n*=30)	**Non-diabetic smokers** (*n*=32)	**Non-diabetic non-smokers** (*n*=30)
Candida albicans	26 (78.8%)*†	24 (80%)	21 (65.6%)*	8 (26.8%)†
Candida tropicalis	5 (15.1%)	4 (13.3%)	4 (15.6%)	4 (13.3%)
Candida albicans+Candida tropicalis	2 (6.1%)	2 (6.7%)	6 (18.8%)	4 (13.3%)
Candida parapsilosis	—	—	—	1 (3.3%)
No *Candida* species isolated	—	—	—	13 (43.3%)

* P<0.05 † P<0.05 ‡ P<0.05

isolated from 15.5% smokers and 13.3% non-smokers with T2DM. Among subjects without T2DM, *C. tropicalis* was isolated from 15.6% smokers and 13.3% non-smokers. *C. tropicalis* carriage was comparable among smokers and non-smokers with and without T2DM (*Table 2*). In 43.3% non-diabetic non-smokers, no yeast species were identified. None of the study participants harbored other oral *Candida* species (*Candida krusei*, *Candida glabrata*, *Candida luscitanie* and *Candida gullerimondi*).

Discussion

The present study was based on the hypothesis that oral *Candida* carriage is higher in type 2 diabetic smokers as compared with type 2 diabetic non-smokers. Interestingly, the present results showed comparable outcomes in terms of oral *Candida* carriage among type 2 diabetic smokers and non-smokers. An explanation in this regard may be derived from the fact that in the present study, all patients with T2DM (regardless of their smoking status) were hyperglycemic (*Figure 1*). It has been reported that the salivary glucose levels are significantly higher in patients with T2DM as compared with non-diabetic controls [23]. Salivary glucose forms chemically reversible glycosylation products with proteins in tissues in hyperglycemic patients, which leads to the accumulation of glycosylation products on oral epithelial cells [24]. This may in turn increase the number of available receptors for *Candida* on oral tissues. This could have favored the adhesion and growth of *Candida* species on the tongue and oral epithelia of patients with T2DM and the contribution of tobacco smoking seems to be secondary. Moreover, it is also noteworthy that type 2 diabetic smokers and non-smokers had significantly lower salivary flow rates as compared with non-diabetic participants. Moreover, a persistent dry oral environment in patients with T2DM could have facilitated the stagnation and proliferation of oral *Candida* species. It is also

possible that there may be intrinsic qualitative changes on the cell surface receptors modulating the adhesion of oral *Candida* species in patients with T2DM [25]. Furthermore, according the present results, none of the patients with T2DM reported to brush the dorsum of the tongue as a regular or irregular oral hygiene maintenance regimen. This is yet another factor could have been associated with comparable oral *Candida* carriage among smokers and non-smokers with T2DM. These results suggest the oral *Candida* carriage in patients with T2DM is mainly governed by hyperglycemia and the role of smoking in this regard is rather secondary. In the present study, *C. albicans* was more often isolated from the oral cavity of non-diabetic smokers as compared with non-smokers. These results are in accordance with earlier reports [26-29]. Various explanations have been posed in this regard. It is well-known that tobacco smoking obstructs neutrophil transmigration across the oral and periodontal microvasculature, suppresses neutrophil cell spreading, chemotaxis and phagocytosis and results in protease release from neutrophils that may significantly contribute in tissue destruction [30]. Tobacco smoking has also been reported to augment oral *Candida* carriage by increasing epithelial keratinization [31], reducing salivary immunoglobulin A levels [32] and suppressing the function of polymorphonuclear leukocyte [33].

There are a few limitations of the present study. In the present study, FBG levels were used to monitor glycemic levels in the patient population. It is known that measurement of hemoglobin A1c (HbA1c) levels reflect the average blood glucose levels of an individual during the past 3 months [17,34]. In the present study, all patients with T2DM were hyperglycemic. Further studies using HbA1c as an essential blood glucose monitoring tool are therefore warranted. It is tempting to speculate that oral *Candida* carriage and species prevalence is lower in patients with well-controlled T2DM (FBG levels 70-90 mg/dl) as compared with patients with poorly controlled T2DM.

This could possibly occur due the presence of significantly lower levels of glucose in patients with well-controlled T2DM as compared with that in patients with poorly-controlled diabetes. Another limitation of the present study is that there was no significant difference in the number of cigarettes smoked daily and duration of smoking habit among tobacco smokers with an without T2DM. It is possible that oral *Candida* carriage may have been higher in type 2 diabetic smokers in case these individuals were smoking significantly greater quantities of cigarettes daily for longer durations than non-diabetic smokers. Further studies are warranted in this regard.

In the present study ~17% non-smokers among patients without T2DM reported to occasionally brush the dorsum of tongue during routine oral hygiene maintenance. This factor may have played a role in reducing oral *Candida* carriage in these individuals as compared with smokers in the same group. It is speculated that tongue brushing as an adjunct to conventional daily oral hygiene maintenance regimes helps reduce oral *Candida* carriage in smokers and non-smokers with and without T2DM. Furthermore, it is highly recommended that additional studies, with a larger sample size be performed in order to control the confounding the confounding factors (such as tooth/tongue brushing and UWSFR) that could have biased the present results.

Conclusion

In patients with T2DM, oral *Candida* carriage and species prevalence is governed by hyperglycemia and the role of tobacco smoking in this regard seems to be rather secondary.

Conflict of Interest and Financial Disclosure

The authors declare that they have no conflict of interest and there was no external source of funding for the present study.

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