Association between glycemic status and oral Candida carriage in patients with prediabetes

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Objective. This study assessed the association between glycemic status and oral Candida carriage among patients with prediabetes.

Study Design. This was a comparative study of oral Candida carriage among individuals with prediabetes. Oral yeast samples were collected from 150 individuals: group A was 43 patients with prediabetes (fasting blood glucose levels and hemoglobin A1c, 100 to 125 mg/dL and ≥5%, respectively); group B was 37 individuals previously considered prediabetic but having fasting blood glucose levels <100 mg/dL and hemoglobin A1c <5%; and group C was 70 medically healthy individuals. Oral yeasts were identified using standard techniques. Unstimulated whole saliva flow rate and number of missing teeth were recorded.

Results. Oral Candida was isolated from 100% of patients with prediabetes and from 65.7% of control participants. Candida albicans carriage was higher among patients with prediabetes (48.7%) (P < .01) and patients in group A (51.2%) (P < .01) than among controls (25.7%). Candida carriage, unstimulated whole salivary flow rate, and number of missing teeth were similar in groups A and B.

Conclusions. Oral Candida carriage was higher in patients with prediabetes than in controls and was independent of glycemic status in patients with prediabetes. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:53-58)

It is well known that immunosuppression influences oral Candida carriage.1-4 Studies1-3 have reported that oral carriage of Candida species, predominantly Candida albicans (C albicans), is higher in patients with poorly controlled diabetes compared with healthy controls. An explanation may be derived from the fact that xerostomia (due to reduced unstimulated whole salivary flow rate [UWSFR]) in patients with poorly controlled prediabetes provides a platform for Candida stagnation and growth on oral tissues, primarily the dorsum of the tongue.1,5 In addition, a high prevalence of Candida species has also been reported in periodontal sites among patients with prediabetes with chronic periodontitis compared with healthy controls.6 It is pertinent to mention, however, that previous studies1,2,5,6 in which oral Candida carriage was investigated in hyperglycemic patients were performed in patients with poorly controlled diabetes mellitus. Prediabetes, a state of impaired glucose tolerance (IGT), is characterized by IGT (140 to 199 mg/dL), impaired fasting glucose (100 to 125 mg/dL), or both.7 In addition, a hemoglobin A1c (HbA1c) test is a useful diagnostic test that correlates with the average blood glucose levels over the past 3 months.8 Individuals with HbA1c levels between 5.5% and 6.4% are categorized as individuals with prediabetes.9

Periodontal inflammation has been reported to be worse in patients with prediabetes compared with healthy controls9,12; it has been hypothesized that

Statement of Clinical Relevance

Oral Candida carriage is similar in patients with prediabetes and in individuals previously diagnosed with prediabetes but having normal blood glucose levels due to glycemic control. This indicates that individuals in either group are equally susceptible to oral Candida infections and that their susceptibility is higher than that of controls.
besides the oxidative stress induced by chronic hyperglycemia, a reduced UWSFR in patients with prediabetes may also have contributed in worsening periodontal status. Because xerostomia is a common manifestation in patients with chronic hyperglycemia, it is assumed that oral Candida carriage is also high in patients with prediabetes compared with healthy controls. However, to our knowledge from indexed literature, oral Candida carriage in patients with prediabetes has not yet been investigated. Furthermore, studies have shown that glycemic control reduces the severity of periodontal inflammation in patients with poorly controlled diabetes and prediabetes. We therefore hypothesized that oral Candida carriage would be reduced in patients previously diagnosed with prediabetes but who maintain their fasting blood glucose level (FBGL) within normal limits (70 to 100 mg/dL) as compared with patients with poorly controlled prediabetes (100 to 125 mg/dL). To our knowledge from indexed literature, this hypothesis had not been tested before our study.

**METHODS**

**Ethical approval**

The study was approved by the research ethics review committee of the Jinnah Postgraduate Medical Center, Karachi, Pakistan. The study was performed in accordance with the Declaration of Helsinki as revised in 2000. It was mandatory for all study participants to have read and signed the consent form before being included in this study.

**Inclusion and exclusion criteria**

Only individuals with medically diagnosed prediabetes (FBGL, 100-125 mg/dL [5.6-6.9 mmol/L]; hemoglobin A1c [HbA1c], 5.7%-6.4%) were included. Exclusion criteria were (1) tobacco smoking; (2) alcohol consumption; (3) exclusive areca nut and gutka chewing; (4) use of antibiotics, antifungal agents, steroids, or nonsteroidal anti-inflammatory drugs within the past 3 months; (5) self-reported systemic diseases, including type 1 and type 2 diabetes mellitus, hepatitis B, hepatitis C, and infection with HIV or AIDS; and (6) wearing partial or complete dentures.

**Study participants**

Patients with prediabetes were recruited from the diabetes care unit of a local hospital in Karachi, Pakistan. Medical records of these patients were explored to confirm the diagnosis of prediabetes. Control participants self-reporting as not prediabetic were recruited from a residential area near the hospital. All participants were invited to an oral health care center in the early morning hours (in a fasting state) for FBGL measurement and collection of oral yeast and unstimulated whole saliva (UWS) samples.

**Hemoglobin A1c levels and fasting blood glucose levels**

Hospital records of patients with prediabetes were searched to determine the most recent HbA1c levels. A digital glucometer (Accu-Chek Activ, Roche Diagnostics, Mannheim, Germany) was used to measure the FBGL. Depending on the glycemic levels, patients with prediabetes were divided into 2 subgroups as follows: group A, patients with prediabetes with FBGL between 100 and 125 mg/dL (HbA1c ≥5%), and group B, patients with prediabetes with FBGL <100 mg/dL (HbA1c <5%). Self-reported systemically healthy individuals (FBGL, 70 to <100 mg/dL) were categorized as controls (group C).

**Collection of UWS samples**

To collect the UWS samples, participants were seated comfortably in a chair in a “coachman” position and requested to spit (without swallowing) into a gauged measuring cylinder for five continuous minutes. UWSFR was recorded in milliliters per minute (mL/min).

**Collection of oral yeast samples**

Oral Candida samples were collected as described previously. In summary, each sample was collected by scraping the dorsum of the tongue and bilateral buccal mucosa with a sterile cotton swab (bioMérieux SA, Montalieu-Vercieu, France). Immediately after sampling, the swabs were returned to the containment tube to avoid contamination. At 37°C, Candida strains were cultured in Sabouraud dextrose agar (Becton, Dickinson and Company, Sparks, MD, USA) to quantify the colony-forming units in the oral cavities of individuals with and without prediabetes. After 24 hours, all cultures were inspected, and monitoring continued until 7 days of incubation for yeast growth, following which they were subjected to speciation.

**Identification of oral yeast samples**

A yeast identification system (API 32-C System yeast identification programme, bioMérieux) was used to identify the oral yeast species. Yeast isolates that could not be identified with the oral yeast identification system were subjected to molecular identification. Molecular identification was performed as described elsewhere. Briefly, for DNA isolation, yeast cells were suspended in 200 μL sterile polymerase chain reaction (PCR)–grade water, and genomic DNA was prepared using a DNA preparation robot (Roche Diagnostics GmbH, Mannheim, Germany). Using
universal primers and ampliTaq Gold DNA polymerase for DNA sequencing and PCR analysis (Applied Biosystems, Foster City, CA), a region of about 500 base pairs (bp) of 18S ribosomal ribonucleic acid gene was amplified by PCR. Primers and free nucleotides from the PCR products were removed using the 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 a genetic analyzer (ABI 310; Applied Biosystems, Foster City, CA, USA). Both strands of PCR amplified DNA fragments were sequenced in order to shun error of sequencing. The DNA sequence was analyzed and searched in the Blast DNA database for yeast identification and typing.

Questionnaire
A standardized questionnaire was administered to all participants by one investigator (A.S.). The questionnaire comprised the following questions: “(1) What is your age (in years)? (2) What is your gender (male/female)? (3) Do you have prediabetes (yes/no)? (3a) If yes, since when do you have prediabetes? (3b) What type of treatment has your doctor recommended for the management of prediabetes (allopathic, herbal, dietary control, others)? (4) Do you brush your teeth (yes/no)? (4a) If yes, then how many times do you brush your teeth ([i] once a day, [ii] two times a day, [iii] three times a day, [iv] more than three times a day)? (5) If you do not brush your teeth every day then how often do you brush your teeth (specify)? (6) Do you brush your tongue (yes/no)? (6a) If yes, then how many times do you brush your tongue every day ([i] once a day, [ii] two times a day, [iii] three times a day, [iv] more than three times a day)? (7) Do you rinse your mouth with an oral rinse or mouthwash (yes/no)? (7a) If yes, then how many times do you rinse your mouth with an oral rinse or mouthwash ([i] once a day, [ii] two times a day, [iii] three times a day, [iv] more than three times a day)"

Oral lesions, lesions on the tongue, and number of missing teeth
In all groups, one examiner (A.M.) performed clinical diagnosis of lesions on the buccal vestibule and tongue (coated tongue, fissured tongue, hairy tongue, geographic tongue, and median rhomboid glossitis [MRG]) using standardized World Health Organization criteria. In all groups, the number of missing teeth (MT) (excluding third molars) were counted by the same investigator (A.M.).

Statistical analysis
Data were statistically analyzed using SPSS software (version 18, SPSS Inc, Chicago, IL, USA). Level of significance between the groups (groups A, B, and C) was assessed using Mann-Whitney U test. For multiple comparisons, the Bonferroni post hoc test was used. Level of significance was set at $P < .05$. A multiple logistic regression model was applied to adjust for confounding variables (age, gender, number of MT, oral hygiene measures, UWSFR, and culture/PCR results).

RESULTS
Characteristics of the study cohort
Eighty patients with prediabetes (43 patients [38 males and 5 females] in group A and 37 patients [35 males and 2 females] in group B) and 70 controls (61 males and 9 females) were included for study. There was no significant difference in age among participants in groups A (41.2 ± 1.6 years), B (43.1 ± 2.1 years), and C (40.6 ± 1.5 years). The mean duration of prediabetes among participants in groups A and B was 11 ± 2.2 months and 13.2 ± 1.4 months, respectively (Table I).

The mean FBGL was significantly higher among the population with prediabetes (109.3 ± 4.2 mg/dL) and patients in group A (119.3 ± 3.5 mg/dL) than among individuals in the control group (78.6 ± 0.7 mg/dL) ($P < .05$), respectively. Mean FBGL was significantly higher among patients with prediabetes in group A (119.3 ± 3.5 mg/dL) than in group B (88.6 ± 2.2 mg/dL) ($P < .05$). Among patients with prediabetes, mean HbA1c levels were significantly high in group A (6.2 ± 0.5%) than in group B (4.9 ± 0.3%) ($P < .01$) (see Table I). HbA1c levels among patients with prediabetes in group A and group B were measured 44.5 ± 4.6 days and 11.3 ± 2.4 days, respectively, prior to the present investigation.

On clinical examination, none of the participants displayed tongue lesions, and there was no significant difference in the number of MT and UWSFR in individuals with and without prediabetes (see Table I).

Oral Candida carriage
Oral C. albicans carriage was significantly higher in the population with prediabetes ($n = 80$) (48.7%) and the patients with prediabetes in group A (51.2%) compared with group C ($n = 70$) (25.7%) ($P < .01$). There was no difference in carriage of Candida tropicalis (C. tropicalis), Candida parapsilosis (C. parapsilosis), and C. albicans + C. tropicalis (as mixed species) among patients with prediabetes and individuals in group C. Among patients in groups A and B, there was no significant difference in oral carriage of C. tropicalis, C. parapsilosis, and C. albicans + C. tropicalis. C. albicans + C. parapsilosis as mixed species and Candida krusei were isolated from 2.9% and 1.4% of individuals in group C (Table II).

Our multiple logistic regression model showed no significant association between oral Candida carriage
and number of MT, daily oral hygiene maintenance regimens, and UWSFR (data not shown).

Oral and tongue lesions
Oral and tongue lesions (such as coated tongue, fissured tongue, hairy tongue, geographic tongue, and MRG) were not detected in any group clinically examined in this study.

Questionnaire
In group A, 86% (n = 37/43) individuals reported brushing their teeth once daily; whereas in groups B and C, 83.7% (n = 31/37) and 84.3% (n = 59/70) individuals, respectively, reported brushing their teeth once daily. None of the individuals in the study population reported brushing their tongue or using oral rinses or mouthwashes as a component of their oral hygiene maintenance regimens.

DISCUSSION
To our knowledge from indexed literature, this is the first study in which oral Candida carriage was investigated in patients with prediabetes with particular emphasis on glycemic status. In general, the population with prediabetes investigated in the present study was hyperglycemic (FBGL, 109.3 ± 4.2 mg/dL; HbA1c, 5.8 ± 0.2%), which is a possible explanation for the increased oral C. albicans carriage in patients with prediabetes (n = 80) compared with healthy controls (70 individuals in group C). Our findings are in accordance with those of earlier studies, in which oral Candida carriage was reported to be increased in patients with poorly controlled type 2 diabetes as compared with controls.

Glycemic control has been reported to enhance healing and reduce periodontal inflammation in patients with diabetes mellitus and prediabetes. In the present study, we hypothesized that glycemic control reduces oral Candida carriage in patients with prediabetes. Interestingly, the present results showed no significant difference in oral Candida carriage among individuals with prediabetes in Group A and individuals previously prediabetic but now having normal glycemic levels due to dietary control (group B). Various explanations may be proposed to explain these results.

Table II. Oral Candida species isolated from individuals with and without prediabetes

<table>
<thead>
<tr>
<th>Oral Candida species</th>
<th>All patients with prediabetes (n = 80) n (%)</th>
<th>Patients in group A (n = 43) n (%)</th>
<th>Patients in group B (n = 37) n (%)</th>
<th>Individuals in control group (n = 70) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>39 (48.7%)</td>
<td>22 (51.2%)</td>
<td>17 (46%)</td>
<td>18 (25.7%)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>26 (32.5%)</td>
<td>14 (32.5%)</td>
<td>12 (32.4%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>C. albicans + C. tropicalis</td>
<td>13 (16.3%)</td>
<td>6 (14%)</td>
<td>7 (18.9%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>2 (2.5%)</td>
<td>1 (2.3%)</td>
<td>1 (2.7%)</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>C. albicans + C. parapsilosis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>C. lusitaniae*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C. glabrata*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C. krusei*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>C. guilliermondii*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>No Candida species isolated</td>
<td>24 (34.3%)</td>
<td>—</td>
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<td></td>
</tr>
</tbody>
</table>

*These Candida species were identified using polymerase chain reaction.

1p < .01.

2p < .05.

3p < .05.

4p < .01.

5p < .01.

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It is known that aging, increased number of MT, poor oral hygiene maintenance, and xerostomia are significant risk factors for Candida growth on oral tissues, particularly the dorsum of the tongue. In the present study, participants in group A and group B were nearly 40 years old, performed similar daily oral hygiene maintenance protocols, and had no significant differences in UWSFR and number of MT. In addition, the short duration of prediabetes among patients in groups A and B (nearly 1 year) may have been unable to induce significant changes in the periodontal status as well as salivary flow rate in these individuals. Furthermore, it is pertinent to mention that the most recent HbA1c levels among participants in group B were measured nearly 2 weeks before the present investigation. It is tempting to speculate that individuals in group B could have been maintaining glycemic levels since merely 2 weeks, which may have been an insufficient time duration to reduce oral Candida carriage in these individuals as compared with those in group A (in which HbA1c levels were measured nearly 40 days before the present investigation). It is probable that long-term control of hyperglycemia may reduce oral Candida carriage in patients with diabetes and prediabetes; however, further longitudinal studies are warranted in this regard.

A direct association between tongue lesions (including MRG) and oral candidiasis, tobacco smoking, denture wearing, and systemic conditions (such as diabetes mellitus and AIDS) has been reported. Lesions in the oral cavity (particularly those on the buccal mucosae) and tongue lesions (such as hairy tongue, fissured tongue, coated tongue, and MRG) were not detected in any group clinically examined in this study. Although none of the study participants reported brushing the dorsum of the tongue as an adjunct to the regular oral hygiene maintenance regimen, the normal UWSFR that existed in all study groups could have prevented oral Candida species from accumulating and multiplying on the dorsum of the tongue, thereby preventing the occurrence of tongue lesions. Since tobacco smokers and betel nut chewers were excluded from the present study, it is possible that oral and tongue lesions are more common in patients with prediabetes who habitually smoke or chew tobacco products than in those who do not use tobacco in any form.

There are a few limitations of the present study that we address. First, quantification of the oral Candida species was not performed, and this would have been useful for better understanding these data. Second, categorization of the individuals with prediabetes into groups A and B was based on measurement of HbA1c and FBGL levels; whereas glycemic levels in self-reported controls were determined using FBGL alone. It is known that the oral glucose tolerance test (OGTT) is a valuable and reliable tool for monitoring hyperglycemia; therefore, it is highly recommended that OGTT should be considered as a critical parameter in future studies dealing with glycemic status in patients with diabetes and in undiagnosed individuals. Third, tobacco users were excluded from this study, and tobacco smoking is a significant risk factor for an increased oral Candida carriage. It is tempting to speculate that smokers with prediabetes are more susceptible to oral fungal infections (due to an increased oral Candida carriage) as compared with nonsmokers with prediabetes and nondiabetic smokers and nonsmokers. Fourth, most of our study participants were men. It has been reported that oral Candida carriage is significantly higher in women with type 2 diabetes compared with men with type 2 diabetes. Thus, further studies are needed to assess the limitations of the present study.

Within the limits of the present investigation, it is concluded that oral Candida carriage is higher in patients with prediabetes than in controls and may be independent of glycemic status in patients with prediabetes.

REFERENCES


