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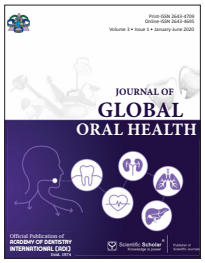

Research Article

Association of diabetes mellitus on the development of oral candidiasis

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ABSTRACT

Objectives: The objective of this study was to determine the association of diabetes mellitus (DM) with the development of oral candidiasis among known diabetic mellitus patients. **Materials and Methods:** A prospective cohort study was conducted using 100 DM subjects with 50 non-diabetic endocrine subjects and 50 apparently healthy nursing staff as comparative groups. An oral rinse, blood, and saliva were obtained for fasting blood glucose test, hemoglobin A1c, and fasting salivary glucose (FSG) tests, respectively. **Results:** Sixty-six DM subjects were positive for *Candida*. Twenty-nine and 23 subjects were positive in the non-diabetic endocrine and the apparently healthy group, respectively. Unlike the comparison group, the total colony-forming units per milliliter of *Candida* were raised in the DM group. Of all *Candida* species isolated, 70.37% of the single-species isolates were *Candida albicans,* and 29.6% were *non-albicans Candida*. *Candida* growth was significantly associated with the food pattern of participants ($P = 0.026$), use of dentures ($P = 0.001$), duration of wearing dentures ($P = 0.006$), and FSG level ($P = 0.026$). A significant correlation was identified between the *Candida* growth and FSG level of participants ($P = 0.026$, r = 0.239), the log value of the total colony count (per mL), and the FSG level of the individuals ($P = 0.029$, r = 0.234), and also between FSG level and fasting blood sugar level of participants ($P = 0.005$, r = 0.297). **Conclusion:** Significantly higher colonization of *Candida* was identified in diabetic patients compared to non-diabetic or healthy individuals. Food pattern, wearing of dentures, and FSG were significant contributing factors for oral *Candida* growth.

Keywords: Oral candidiasis, Diabetes mellitus, Salivary glucose, Dentures

INTRODUCTION

The genus *Candida* encompasses approximately 200 species; however, just a few of these varieties are human pathogenic opportunistic organisms which trigger opportunistic infections when the immune system of the victim is compromised or debilitated. Infections caused by *Candida* can be either superficial or invasive.[1,2] *Candida* is a common inhabitant of the epidermis, digestive, and genitourinary tracts. In addition, *Candida albicans* is the species most commonly isolated from specimens from hospitals, but other species (*non-albicans Candida*) have been increasingly observed.[3]

Unlike bacterial infections, fungal infections tend to develop over a longer period. Extended hospital stays, long-term chronic illnesses, surgery (especially abdominal surgery), burns, long-term stays in an intensive care unit, chronic steroid therapy, previous administration of broad-spectrum antibiotics, antineoplastic chemotherapy, systemic infections such as diabetes mellitus (DM), Addison's

disease, human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and malignancy, immune suppressive conditions, and as risk factors impaired salivary gland functions, drugs, dentures, high carbohydrates diets, smoking have been increased the possibility of development and colonization of fungal infections, oral candidiasis (OC), as well as oral fungal infections.[4-6]

It is projected that almost 1 billion individuals have fungus-related infections of the skin, nails, and hair, tens of millions have mucosal candidiasis and over 150 million people have life-threatening or fatal fungal diseases.[7] DM, a rising global pandemic, as of 2019, had claimed over 460 million lives worldwide. It is estimated to be on an upward trajectory and may reach more than 700 million by the year 2045.[8] The largest-ever study conducted in Sri Lanka in 2005 showed the diabetic prevalence among males to be 14.2% and among females to be 13.5%.[9] DM is a chronic disorder that tends to cause chronic complications, including diabetic neuropathy, diabetic retinopathy, and diabetic nephropathy.[10] Throughout the years, DM has been linked with numerous oral complications such as xerostomia, burning mouth sensation, and the presence of oral opportunistic fungal infections such as OC.[11] Additional factors, such as the use of dentures, might be contributing to this cause.[12]

The present study was conducted to find the association between the development of OC and DM. Biochemical parameters such as fasting blood glucose, hemoglobin A1c (HbA1c), and fasting salivary glucose (FSG) were measured and statistically analyzed to find associations and correlations. The salivary flow rate was also measured and analyzed to determine associations and correlations with OC.

MATERIALS AND METHODS

Study setting

A total of 200 subjects were recruited. Confirmed DM patients (n = 100) aged between 20 and 60 years, confirmed endocrine patients (n = 50) (not suffering from DM) aged between 20 and 60, who were registered at the diabetic and endocrinology clinic, Colombo South Teaching Hospital (CSTH), Kalubowila, Sri Lanka were selected as the patient group and the comparative patient group, respectively. Apparently, healthy nursing staff (n = 50) aged between 20 and 60, who were employed at the time of sampling at the same hospital, were selected as the apparently healthy group.

All study participants were randomly selected for the study after obtaining informed consent and they were requested to attend the next clinic with 8–10 h fasting. DM patients and non-diabetic patients who had undergone specific treatment such as anti-neoplastic therapy, those who are diagnosed with HIV/AIDS and other oral cavity infections, patients above 60 years of age or pregnant, and patients undergoing treatments for any chronic disorders (e.g., thyroid disorders, hypertension, dyslipidemia, and hyperlipidemia) were excluded from the study.

Sample collection

All individuals, including patients and healthy groups, were investigated and confirmed by the medical professionals. Oral rinse samples were collected from all after rinsing their mouth thoroughly using 10 mL of sterile phosphate-buffered saline (1M; pH 7.2) for analysis of the presence of fungi species. Samples were collected in to sterile plastic culture containers, after rinsing the mouth thoroughly for 1 minute.[13] Then, samples were transported to the laboratory within 2 h at 4°C in an iced box. Each oral rinse samples were centrifuged at 5000 rpm for 10 min. The deposit (about 1 microliter amount) was inoculated onto a Sabouraud dextrose agar medium with chloramphenicol and incubated at 35°C for 24 h. *Candida* growth was morphologically identified as smooth, white or creamy-colored buttery colonies. The individual CFUs were counted manually. The samples which were negative for *Candida* growth after the first 24 h of incubation were re-incubated at 35°C for a further 24 h. Suspected *Candida* colonies were further identified using Gram stain and differentiated using germ tube test and the CHROM agar *Candida* (CHROM agar, France) medium. The light green colonies were identified as *C. albicans*, dark green colonies as atypical *C. albicans*, flat pink dry colonies as *Candida Krusei,* and whitish colonies as other *Candida* species.

Salivary samples were collected from each of the 200 subjects (100 DM patients, 50 non-diabetic endocrine patients, and 50 apparently healthy individuals) subsequent to the collection of the oral rinse. The salivary flow rate was estimated after pooling saliva in their mouth for 5 min, and the FSG levels in each individual were also measured. FSG levels were determined on the saliva samples (glucose oxidase method, GOD) by a semi-automated analyzer (Chrome Tech B01-CT-8 series double-beam UV/Vis spectrophotometer). The supernatant from centrifuged (centrifugation at 4500 rpm for 5 min) saliva samples were used for the estimation of glucose levels. A sample of 1.5 mL of fasting venous blood was collected from all subjects (100 DM patients, 50 non-diabetic endocrine patients, and 50 apparently healthy individuals) to analyze Fasting Blood Sugar levels (Konelab 30, GOD method). Each individual HbA1c levels were measured using 1.5 mL of whole blood which was collected into ethylenediamine tetraacetic acid (EDTA) tubes (high-performance liquid chromatography, Bio-Rad D10).

Data analysis

Sociodemographic data, family history, and medical and clinical history of the patients were collected using an

interviewer-administrated questionnaire. Data were analyzed using the statistical software; Statistical Package for the Social Sciences version 23.0. Continuous variables were analyzed and the analyzed data were classified for further analysis. Sociodemographic data (age, gender, marital status, height, weight, body mass index (BMI), food pattern, patient history, denture wearing, smoking, and alcohol use) and results of laboratory investigations were descriptively analyzed. The correlations between variables were analyzed using bi-variant correlations. The mean values of individual data were compared using independent *t*-tests. The associations among the groups mentioned above were then obtained using the Chi-square test.

RESULTS

Descriptive statistics

One hundred DM patients, 50 patients from the endocrinology clinic without diabetes, and 50 individuals who appeared to be healthy were analyzed to determine the association between DM and the development of OC in known diabetic mellitus patients. The sociodemographic data and laboratory investigations of the participants were analyzed, as shown in Table 1. All three study groups represented a female predominance and a mean age of ≥45 years.

DM patients were categorized into age groups and a majority (45%) of patients were in the age range 41–60 years. Further, the BMI category of DM patients was analyzed according to the Asian BMI categorization, and it was identified that 62% of the patients were obese, and only 21% had a normal BMI. According to the medical history of the DM patients, 96% of the individuals were suffering from Type 2 DM, and 4% had Type 1 DM. Among the 23 DM patients wearing dentures, 75% have been wearing them for ≤10 years, and 25% have been wearing them for 11–20 years.

The *Candida* growth of the DM patients was compared between study groups. Fungal growth was given for oral rinse samples grown on sabouraud dextrose agar. According to the *Candida* growth, 66 (66%) individuals out of 100 DM patients showed positive growth. Among the individuals who had positive *Candida* isolates, certain individuals had only one species, while other individuals had two species isolated [Table 2]. Importantly, the number of colonies isolated in the oral cavity showed a significant variation among the three study groups [Table 3]. Diabetic patients who gave positive growth in the study group were altogether 66. Greater than 10000 CFU/mL colony count were identified in 41 patients (62.12%), which is significantly higher comparatively to other study groups.

The following comparisons were done using the independent *t*-test [Table 4]. *Candida* growth was significantly associated with the food pattern of the participants ($P = 0.026$). Non-vegetarians had a comparatively higher *Candida* growth than vegetarians. Wearing of dentures ($P = 0.001$) and duration of wearing dentures ($P = 0.006$) also had a significant association with the growth of *Candida* in the oral cavity. Participants wearing dentures and, more significantly who have been wearing them for more than 10 years had a comparatively higher probability of having *Candida* growth. Moreover, FSG level had a significant association with *Candida* growth

Table 1: Descriptive statistics of sociodemographic data and laboratory investigations.

Characteristics/Investigations	DM patients	Non-diabetic endocrine patients	Apparently, healthy nursing staff
	Mean (SD), percentage	Mean (SD), percentage	Mean (SD), percentage
Age (years)	56.8 (2.3)	45 (11.7)	45.2 (8.4)
Gender			
Male [*n*]	34% [34]	10% [05]	12% [06]
Female [*n*]	66% [66]	90% [45]	88% [44]
Weight (kg)	62.6 (11.8)	62.4 (13.9)	63.4 (10)
Height (cm)	154.9 (9.7)	152.6 (7.1)	157.3 (7.3)
BMI	26.1 (4.4)	26.8 (5.7)	25.7 (4.3)
Food pattern			
Vegetarian [*n*]	5% [5]	6% [3]	2% [1]
Non-vegetarian [*n*]	95% [95]	94% [47]	98% [49]
Wearing dentures			
Yes [*n*]	23% [23]	12% [6]	8% [4]
No [*n*]	77% [77]	88% [44]	92% [46]
Fasting blood glucose (mg/dL)	140.5 (41.4)	94.1 (14)	90.4 (8.0)
HbA1c (%)	8.5 (1.7)	5.7 (0.4)	5.5 (0.4)
Fasting salivary glucose (mg/dL)	1.3 (1.3)	0.8 (0.7)	0.6 (0.4)
Salivary flow rate (mL/5 min)	2.3 (1.1)	2.6 (1.6)	2.8 (1.5)

BMI: Body mass index, HbA1c: Hemoglobin A1c, SD: Standard deviation, DM: Diabetes mellitus

(P = 0.026), where individuals with an FSG level ≥4.1 mg/dL were identified to be having a greater possibility for *Candida* growth.

A significant correlation was identified between the *Candida* growth and the FSG level of participants (P = 0.026, r = 0.239). The log value of the total colony count (per mL) had a significant correlation with the FSG level of the individuals (P = 0.029, r = 0.234). Furthermore, FSG level presented a significant correlation with the fasting blood sugar level of participants (P = 0.005, r = 0.297). Each variable was statistically analyzed against *Candida* growth, and significant associations were entered into a regression to obtain a model. Accordingly, a model with a higher significance of 0.001 was obtained. Food pattern, wearing of dentures, and FSG were identified to be significant contributing factors affecting the oral growth of *Candida*.

DISCUSSION

DM is a metabolic condition characterized by persistent hyperglycemia and impaired glucose homeostasis due to beta-cell malfunction in the pancreatic islets. Fungal infections are more common in people with DM, likely due to immunological disturbances and changes in the chemical makeup of their saliva. Nutrients are supplied for excessive *Candida* growth through high glucose levels in blood and saliva. People with diabetes have an increased risk of developing oral lesions due to *Candida* infection, such as denture stomatitis, pseudomembranous candidiasis, median rhomboid glossitis, and angular cheilitis. In addition, systemic drugs commonly used by people with diabetes tend to decrease saliva production, which, in turn, promotes the growth of germs in oral biofilms and increases the risk of infection. Oral yeast infections affect about 30% of people with diabetes at some time in their lives.[14] Thus, the present study was conducted to evaluate the association of DM on the development of OC and to identify possible risk factors and other associated variables. Several other studies, however, have demonstrated that the likelihood of *Candida* growth in diabetic patients is substantially greater than in healthy non-diabetic individuals.[12,15] Meanwhile, colony count or *Candida* colonization was considerably higher in diabetic patients than in non-diabetic or healthy individuals. The populations of the investigations conducted by Sampath *et al*. and Chouhan *et al*. also revealed this fact.[16,17] Moreover, the co-occurrence of multiple *Candida* species was a prevalent finding of the present investigation and the most common species of fungi found in cultures was *C. albicans*.

Statistical analysis was used to portray the relationship between the influencing factors and the growth of *Candida*. *Candida* proliferation in the oral cavity was statistically proven to be directly influenced by dietary pattern, denture wear, denture wear duration, and FSG level. Importantly, the application of dentures and the duration of denture wear were

Table 2: Growth of *Candida* and species percentages among three study groups.

	Diabetic patients *n* (%)		Non-diabetic endocrine patients *n* (%)		Apparently, healthy nursing staff *n* (%)	
Growth positive	66 (66)		29 (58)		23 (46)	
One species						
C. albicans	37 (70.37)	54 (54.0)	19 (70.37)	27 (54.0)	20 (95.24)	21 (42.0)
Atypical *C. albicans*	6 (9.26)		-		-	
C. krusei	-		-		-	
Other *Candida* spp.	11 (20.37)		8 (29.63)		1 (4.76)	
Two species						
C. albicans and Atypical *C. albicans*	7 (58.33)	12 (12.0)	1 (50.00)	2 (4.0)	-	2 (4.0)
C. albicans and C. krusei	2 (16.67)		1 (50.00)		-	
C. albicans and other *Candida* spp.	2 (16.67)		-		1 (50.00)	
Atypical *C. albicans* and *C. krusei*	1 (8.33)		-		1 (50.00)	

C. albicans: Candida albicans, C. krusei: Candida krusei

Table 3: Variations of colony count among three study groups.

Colony count per milliliter	Diabetic patients (*n*=66) %	Non-diabetic endocrine patients (*n*=29) %	Apparently, healthy nursing staff (*n*=23) %
1000–2000 CFU/mL	7 (10.61)	12 (41.38)	4 (17.39)
2000–5000 CFU/mL	12 (18.18)	4 (13.79)	11 (47.83)
5000–10000 CFU/mL	6 (9.09)	3 (10.35)	4 (17.39)
>10000 CFU/mL	41 (62.12)	10 (34.48)	4 (17.39)

Table 4: Mean comparisons among variables.

Study group	Mean	SD	*P*-value
FBG			
Diabetic patients	140.50	41.40	0.001
Apparently healthy nursing staff	90.40	8.00	
HbA1c			
Diabetic patients	8.45	1.68	0.001
Apparently healthy nursing staff	5.50	0.360	
FSG			
Diabetic patients	1.33	1.25	0.001
Apparently healthy nursing staff	0.60	0.43	
Salivary flow rate			
Diabetic patients	2.28	1.11	0.027
Apparently, healthy nursing staff	2.76	1.47	
Total colony count			
Diabetic patients	26240.000	43676.06	0.001
Apparently, healthy nursing staff	5100	19421.50	
FBG			
Diabetic patients	140.50	41.40	0.001
Non-diabetic endocrine patients	94.08	13.97	
HbA1c			
Diabetic patients	8.45	1.68	0.001
Non-diabetic endocrine patients	5.74	0.42	
FSG			
Diabetic patients	1.33	1.25	0.007
Non-diabetic endocrine patients	0.79	0.71	
Salivary flow rate			
Diabetic patients	2.28	1.11	0.127
Non-diabetic endocrine patients	2.62	1.56	
Total colony count			
Diabetic patients	26240.000	43676.06	0.254

FBG: Fasting blood glucose, HbA1c: Hemoglobin A1c, FSG: Fasting salivary glucose, SD: Standard deviation

identified as significant contributors to the development of *Candida* in the oral cavity of diabetic patients. Several studies have demonstrated that wearing dentures is a significant risk factor for OC, particularly in diabetic patients.[18] Comparable to the present study, Ganapathy *et al.* discovered a positive correlation between OC and denture utilization in diabetic patients.[19] The duration of denture use was associated with fungal proliferation. Long-term use of dentures has increased oral *Candida* development. The presence of fungal spores, conidia, and infrequent denture cleansing would impact the presence of *Candida* species. The composition of a patient's used dentures may have a direct effect on the proliferation and type of fungi.[20] Cleaning dentures by the user and cleaning materials/solutions may also inhibit fungal growth. Materials of dentures and denture cleansing solutions can be examined to comprehend the impact of dentures on fungus growth.[21] The present study depicted that the food pattern of participants has a significant contribution to OC. Specifically, non-vegetarians had comparatively high possibility for candidiasis compared to vegetarian individuals. In contrast, Patil *et al*. reported that the prevalence of *Candida* was higher in vegetarians than in non-vegetarians and that a vegetarian diet predisposed participants to a higher prevalence of *Candida*.[22] Several factors, including a decreased salivary flow, a low pH, and a diminished buffering capacity, may have contributed to the enhanced sustenance of *Candida* in vegetarians.[22] Further research can be conducted to identify variations in fungal/*Candida* growth in relation to the meal/food pattern consumed by patients. The acidity/alkalinity of food may inhibit the proliferation of fungi in the oral cavity of diabetic and non-diabetic endocrine patients. After a meal, the pH that remains in the oral cavity throughout the day affects the proliferation of fungi. The frequency with which participants brushed their teeth, as well as the extent to which they cleaned their teeth, tongue, buccal mucosa, hard palate, gingiva, and the floor of their mouths, would influence the growth and type of fungi.[23]

Non-diabetic endocrine patients can be screened for different kinds of endocrine disorders to identify the influence related to each hormone. Further studies can be carried out to identify the level of insulin hormone presence in blood/saliva in diabetic mellitus patients.

CONCLUSION

The present study identified significantly higher colonization of *Candida* in diabetic patients compared to non-diabetic or healthy individuals. The level of FSG and salivary flow rate presented a significant difference among diabetic and healthy individuals. Importantly, food pattern, wearing of dentures, and FSG were identified to be significant contributing factors affecting the oral growth of *Candida*.

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Ethical approval

The study was commenced after obtaining ethical clearance from the Ethical Review Committee at the CSTH Kalubowila (Application No. 654, Approval date: 22.05.2018). Participants were enrolled in the research after receiving their permission in writing.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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