

ORIGINAL ARTICLE

A comparative study of oral candidal species carriage in patients with type1 and type2 diabetes mellitus

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ABSTRACT

Context: Diabetes mellitus can have profound effects upon the oral tissues especially in patients with poor glycemic control being prone to severe and/or recurrent infections particularly candidiasis. The main aim was to study the association between Type 1 and Type 2 diabetes mellitus and candidal carriage. **Materials and Methods:** The study design comprised of previously diagnosed 30 patients each with type 1 diabetes mellitus (Group A) and type 2 diabetes mellitus (Group B) and 30 age-, sex- and dental status-matched healthy non-diabetic individuals as controls (Group C). The saliva samples were collected and inoculated onto Sabouraud dextrose agar (SDA) and chromogenic agar culture medium. Candidal colony forming units per ml (CFU/ml) values were determined. **Statistical Analysis:** Data were analyzed by χ^2 test, Mann-Whitney U-test, Spearman's rank correlation and Karl Pearson's correlation coefficient. **Results:** Data analysis showed statistically significant higher positive candidal growth in Group A and Group B when compared to Group C. The CFU/ml values were significantly higher in Groups A and B as compared with Group C. Significant positive correlation of CFU/ml with fasting blood sugar level and HbA1c% in both Groups A and B was seen. Oral signs and symptoms observed in diabetics were dry mouth, burning sensation, fissuring and atrophic changes of tongue and erythematous areas, which positively correlated with candidal load. **Conclusion:** The glycemic control status of the diabetic patients may directly influence candidal colonization. The quantitative and biochemical characterization allows better insight into the study of association of diabetes mellitus and candida.

Key words: Candida carriage, Candidal colony forming units per ml, diabetes mellitus

INTRODUCTION

The prevalence of health problems associated with chronic metabolic diseases represents a challenge to the oral pathologist who frequently has the particular blend of clinical, histopathologic and basic research skills with which to investigate the effects of such diseases on the oral environment

utilizing a variety of parameters.^[1] Early diagnosis of systemic diseases like diabetes mellitus (DM) can be done by correlating the oral symptoms and clinical features of these diseases.^[2]

DM is a highly prevalent worldwide, multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues.^[3] Patients with poor glycemic control are being particularly prone to severe and/or recurrent bacterial or fungal infections.^[4] Some of the early, non-specific signs of uncontrolled diabetes include oral candidiasis and other opportunistic fungal infections.^[2] Candidiasis is the most common mycosis of the mouth in both healthy and immunodeficient persons. It is a superficial opportunistic infection, essentially facilitated by local and systemic predisposing factors. One reason for commonality

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of this disease is probably because 40-60% of healthy adults harbor commensal candida in the oral cavity, without any signs or symptoms of candidiasis.^[5] A number of factors have been associated with oral carriage of yeasts in diabetic patients, such as the type and duration of the disease, the degree of glycemic control,^[6] and denture wearing.^[7]

The study focuses on the relationship between oral candida and diabetes and also to quantify the organisms and to identify oral disease factors, systemic disease factors, or both that promote fungal overgrowth and establish clinical infection (candidiasis). The role of an oral medicine physician is critical in making the early diagnosis, counseling the patient on the importance of diabetes control and referring the patient for further management thereby improving the outcome of patients with DM as oral candidiasis is common in those having undiagnosed or uncontrolled diabetes.^[8] This study aims to study oral candidal species carriage, clinical infection with candida in patients with type 1 and type 2 DM and also to compare the oral candidal species carriage of both type 1 and type 2 DM patients with healthy control subjects.

MATERIALS AND METHODS

This case control study comprised of 30 patients each with previously diagnosed type 1 DM (Group A) and type 2 DM (Group B) and 30 healthy non-diabetic individuals matched for age, sex and dental status as controls (Group C). The exclusion criteria included patients receiving radiotherapy, those under long-term local and systemic drug therapy, those receiving steroid therapy, those diagnosed with malignancy, HIV-positive individuals, hypertensive patients and patients with systemic illness.

The saliva samples were collected using oral rinse technique^[9] during early morning after the patients thoroughly rinsed the oral cavity using 5 ml of sterile phosphate-buffered saline solution (0.1 M, pH 7.4) for 60 seconds. The sample was collected in a sterile screw-capped universal container and stored at 4°C in a refrigerator till transported to the microbiology laboratory for further analysis. Samples were centrifuged at 5,000 rpm for half an hour and re-suspended in 5 ml of sterile normal saline. Five microliters of each sample using a sterile platinum loop were inoculated onto Sabouraud dextrose agar (SDA) and chromogenic agar culture medium (HiCrome Candida Differential HiVeg™ agar base, modified, HiMedia Labs, India) and were incubated at 37°C for a period of 48-72 hours. The presence of candida was confirmed by colony characteristics on SDA and Gram staining and periodic acid Schiff's (PAS) staining in addition to pigmentation characteristics on chromogenic agar. The colonies formed were counted using a magnifying glass and Gallenkamp colony counter. Candidal colony forming units per ml (CFU/ml) values were determined using the following formula, Total CFU/ml = Number of colonies × dilution factor/volume of per sample collected.

The entire procedure was explained to all the participants and informed consent was obtained from them. A thorough clinical and oral examination was performed, details of which along with the biochemical values obtained from patient records, demographic information, assessment of dry mouth^[10,11] and diabetic history were recorded on a proforma.

Ethics

The study was approved by the ethical committee of the institution and is in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975 that was revised in 2000.

Statistical analysis

Data were analyzed by χ^2 test, Mann-Whitney U-test, Spearman's rank correlation and Karl Pearson's correlation coefficient presented through tables and diagrams. All these tests were performed by using SPSS 11.0 version computer software for Windows. Statistical significance was considered at $P < 0.05$ and highly significant at $P < 0.001$.

RESULTS

Of the 30 subjects in each group, positive growth of candidal colonies on SDA and chromogenic agar culture media was demonstrated in 30% ($n = 9$) of Group A, 33% ($n = 10$) of Group B and 7% ($n = 2$) of Group C. Group A and Group B showed statistically significant candidal growth when compared to Group C ($P < 0.05$) [Table 1].

The mean candidal CFU/ml in all positive subjects in Group A, Group B and Group C were 3022.22 ± 3323.31 (SD), 29540.00 ± 35880 (SD) and 6.00 ± 2.83 (SD), respectively [Table 2]. The CFU/ml values of Groups A and B as compared with Group C were statistically significant with P values 0.0339 and 0.0317, respectively.

The mean HbA1c% and fasting blood sugar (FBS) levels in Group A were 8.5% and 129.51 mg/100 ml, whereas in Group B it was 9.15% and 123.5 mg/100 ml, respectively. CFU/ml positively correlated with FBS level and HbA1c% ($r = 0.5709$ and $r = 0.5955$, respectively) in Group A and Group B combined and was statistically significant. But negative correlation was seen with that of duration of the disease in years ($r = -0.0580$) [Table 3].

Oral signs and symptoms in diabetes like erythematous areas in oral cavity, fissured tongue, atrophic changes in tongue and burning sensation were seen to be higher in Group B patients when compared to Group A, except for 83% of Group A patients presented with complications of dry mouth when compared to 67% in Group B [Table 4].

Pseudomembranous candidiasis was observed in 5% ($n = 3$) [Figure 1] and angular cheilitis was seen in 3.33% ($n = 2$) of the total diabetic study population positively correlated with candidal load as determined by CFU/ml of oral rinse and mean HbA1c% in both groups.

A significant positive correlation was observed between CFU/ml and various oral manifestations like presence of erythematous areas ($P=0.3450$), burning sensation ($P=0.0862$) and dry mouth ($P = 0.0862$) [Table 5].

Table 1: Distribution of study samples according to groups and candidal growth

Groups	Positive growth	%	Negative growth	%	Total
A	9	30.00	21	70.00	30
B	10	33.33	20	66.67	30
C	2	6.67	28	93.33	30
Total	21	23.33	69	76.67	90

$\chi^2=7.0810$, $df=2$, $P=0.0290^*$

*Significant 5% level of significance ($P<0.05$)

Table 2: Comparison of study groups with respect to CFU/ml by Mann-Whitney U-test

Group	Mean	SD	Z-value	P value
A	3022.22	3323.31	-1.7146	0.0864
B	29540.00	35880.92		
A	3022.22	3323.32	-2.1213	0.0339*
C	6.00	2.83		
B	29540.00	35880.92	-2.1483	0.0317*
C	6.00	2.83		

*Significant 5% level of significance ($P<0.05$), SD: Standard deviation, CFU/ml: Candidal colony forming units per ml

Table 3: Correlation coefficient between duration, FBS and HbA1C% with CFU/ml by Karl Pearson's correlation technique

Group	Variables	CFU/ml
Total (Group A+Group B)	Duration	$r=-0.0580$
	FBS	$r=0.5709^*$
	HbA1c%	$r=0.5955^*$
Group A	Duration	$r=-0.2316$
	FBS	$r=0.3894$
	HbA1c%	$r=0.2569$
Group B	Duration	$r=0.5038$
	FBS	$r=0.7869$
	HbA1c%	$r=0.5038$

*Significant 5% level of significance ($P<0.05$)

Table 4: Comparison of groups A and B with respect to prevalence of different oral manifestations

Type	Erythema	%	Fissured tongue	%	Atrophic tongue	%	Burning sensation	%	Dry mouth	%
Group A	11	36.67	14	46.67	7	23.33	12	40.00	25	83.33
Group B	14	46.67	16	53.33	8	26.67	18	60.00	20	66.67
Total	25	41.67	30	50.00	15	25.00	30	50.00	45	75.00

DISCUSSION

The presence of *Candida albicans* in the oral cavity is not indicative of disease. In many individuals, the presence of *C. albicans* in the oral cavity without any clinical symptoms suggests that it is a minor component of their oral flora.^[12] Microorganisms are continually being removed from the oral cavity by host clearance mechanisms and so, in order to survive and inhabit this ecosystem, *C. albicans* cells have to adhere and replicate. The oral cavity presents a plethora of ligands and many niches to which *C. albicans* adheres and colonizes. In addition, saliva molecules as well as basic proline-rich proteins, promote *C. albicans* adherence by adsorbing to oral surfaces.^[13]

Major local and systemic factors that predispose humans to candidiasis are infections; DM and other endocrine dysfunctions; immunocompromised state (HIV infection); leukemias; lymphomas; iron, folic acid and vitamin deficiencies; denture wearing; administration of hormonal contraceptives, xerostomic drugs, antibiotics, corticosteroids and other immunosuppressive agents; and radiation therapy.^[14,15]

Superficial, systemic infections and oral candidiasis is thought to be more prevalent among individuals with uncontrolled DM. This patient group also presents with complicated course of infection.^[5] A number of factors have been associated with oral carriage of yeasts in diabetic patients, such as the type and duration of the disease and the degree of glycemic control.^[6] Other factors, such as denture wearing, may also contribute to candidal colonization in diabetic patients.^[7,16]

The overall 31% candida carriage in diabetic patients (Groups A and B) in our study is in accordance with the studies done by Lamey *et al.*^[17] Few other studies showed similar findings as well.^[18,19] This observation supports the role of DM, a metabolic disorder as a precipitating factor for increased oral mucosal colonization of candida.

Furthermore, the finding of a 7% prevalence of candida species in the oral cavity of non-diabetic individuals is within the range of 3% to 47% as reported in the healthy adult population previously shown by Samaranayake *et al.*^[20] thus demonstrating candida as commensal organism of the oral microflora.^[12]

In the present investigation, the application of the oral rinse technique, which allows quantification of candidal



Figure 1: Clinical image shows acute pseudomembranous candidiasis in a diabetic patient

Table 5: Correlation coefficient between various oral symptoms with CFU/ml by Spearman’s rank correlation

Variables	CFU/ml in Group A	CFU/ml in Group B	Total (1+2)
Erythema with CFU/ml	$P=0.4125$	$P=0.8001^*$	$P=0.3450$
Fissured tongue with CFU/ml	$P=0.5500$	$P=0.4572$	$P=0.2905$
Atrophic tongue with CFU/ml	$P=0.1304$	$P=0.1782$	$P=0.0898$
Burning sensation with CFU/ml	$P=0.1375$	$P=0.5674^{**}$	$P=0.0862$
Dry mouth with CFU/ml	$P=0.1375$	$P=0.5674^{**}$	$P=0.0862$

*Significant 5% level of significance ($P<0.05$), ** Significant 10% level of significance ($P<0.10$), CFU/ml: Candidal colony forming units per ml

carriage has shown that diabetic patients (Groups A and B), irrespective of the types, carry a greater number of yeasts, as revealed by a significantly larger number of colony forming units on culture. The CFU/ml values of Groups A and B were significantly greater when compared with Group C and this was in accordance with a study done by Epstein, Pearsall and Truelove,^[21] which suggested that CFU estimation of candida may be a useful adjunct in the analysis of candidal carriage and candidiasis.

A negative correlation of duration of the disease in years with the CFU/ml in both groups of diabetics can also be attributed to the better degree of oral hygiene because of improved awareness of the disease.

In the present study, poorly controlled diabetic patients had mean HbA1c $>6.5\%$ when compared to non-diabetic subjects. The estimation of glycosylated hemoglobin provides an accurate and objective measure of glycemic control over past weeks to months (3 months). Highly glycosylated hemoglobin concentration is considered to be an important factor that affects the rate of candidal carriage and subsequent developing of candidiasis in diabetic patients.^[6,22] A recent study elucidated that glycemic control did not play a role in oral candidal carriage in prediabetes, which was contradictory to our findings.^[23] Thus in our study, the spectrum of oral

manifestations and their symptomatology may partially be dependent on candidal density (CFU/ml) and poor glycemic control reflected by HbA1c%.

It was interesting to note in our study that patients with positive candidal growth had higher glycemic index when compared with that of controls which implies that, the glycemic control over a prolonged period influences the disease status of candida and not the mere fasting and postprandial blood glucose levels. A significant positive correlation of CFU/ml with FBS level and HbA1c% was seen in both Groups A and B. Some previous studies have found no such relationship.^[17] Although the increased candidal density has been reported to be associated with increased concentrations of salivary glucose, the correlation between blood glucose and salivary glucose concentration is partially established.^[24] The essential first step in candidal colonization and infection is adhesion of yeast to epithelial cell surfaces. During hyperglycemic episodes, chemically reversible glycosylation products are formed with salivary glucose and proteins in tissues. It is possible that accumulation of such glycosylation products on buccal epithelial cells may increase the number of available receptors for candida.^[5,25]

It is a proven fact that DM disease process affects the salivary gland secretion quantitatively as well as qualitatively.^[10,26] We consider the similar mechanisms might have operated in 75% of the Group A patients making them to experience the feeling of the dryness of the mouth. Ogunbodede *et al.* in their study have reported complaints of dry mouth in 30.8% of diabetics.^[18] Saliva possesses secretory immunoglobulin A (sIgA) and free secretory component, which inhibit candida cell adhesion to the epithelial cells. Therefore, it is likely that a decrease in salivary flow rate consequent to diabetes may further enhance candidal colonization.

Among the diabetic patients, 40% had complaints of dry mouth and 60% had burning sensation, which showed positive candidal growth hinting at the corroborated role of candida in this symptom of burning mouth sensation. This is in agreement with Vitkov *et al.* who demonstrated that the burning mouth sensation (stomatopyrosis) in diabetic patients with candidal colonization occurs via stimulation of capsaicin (vanilloid) receptor by candida metabolites. Further, they also suggested that candida-induced stomatopyrosis should be regarded as a single symptom in type 2 DM.^[27,28]

In accordance to previous studies, the manifestations of erythematous areas, fissuring and atrophic changes [Figure 2] of the tongue are found to be more prevalent in DM.^[26,29] The frequency and symptomatology of these lesions depend on the duration, the glycemic control and the local factors in an individual. Any alteration in these confounding factors would lead to increased colonization of candida causing varied clinical presentation of the aforementioned manifestations.^[26]



Figure 2: Clinical image shows atrophic changes on the tongue of a diabetic patient

CONCLUSION

Both type 1 and 2 DM patients are more predisposed to candidal carriage density. The glycemic control status i.e. HbA1c% of these individuals may directly influence candidal colonization and various oral manifestations and hence their symptomatology. The assessment of long-term glycemic status of diabetics is more reliable than estimating the fasting and postprandial blood glucose levels for establishing the disease status in these patients having predisposing factors for development of candidal infection. The quantitative and biochemical characterization allow better insight in studying the association of DM and candida. The diagnosis and management of increased candidal colonization and clinical candidiasis may be achieved considering both local and systemic factors. Underlying DM may be identified by the presence of oral candidiasis, which serves as a clinical marker. Further studies involving both candidal characteristics (culture and biochemical) and salivary profile of patients with DM may aid in better understanding of the association of this group of mycotic organisms and DM.

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