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ORAL CANDIDAL COLONISATION IN DIABETES MELLITUS AND IDENTIFICATION OF MOST PREVALENT SPECIES USING CHROMAGAR CANDIDA

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ABSTRACT

Aims to compare the frequency and density of oral candidal colonisation in patients with diabetes mellitus with that of non-diabetics, identify most prevalent Candida species colonizing the oral cavity in diabetics and non-diabetics using CHROMagar candida and to know the spectrum of infections by non-albicans species in diabetics with oral candidiasis.A total 175 patient's known type-I and type-II diabetic patients recruited with written consent. Of which male comprises 68 and female was 107 with mean age of male was 54.75 ±12.82 years IQR (50.02-58.36 years) and female mean age was 56.48±10.34 years IQR ;49.68-58.99 years. As per the analysis age was showed to be statistically significant ($p<0.01$) with incidence of Candida for both groups. Different organism were found during culture with good ambient condition, as per the result, the more incidence was found in *Candida albicans* 55 (31.43%) followed by *Candida parapsilosis* 05(2.86%), *Candida dubliniensis* - 04 (2.29%), *Candida krusei* 3(1.71%). *Candida albicans* +*Candida parapsilosis* and Candida *tropicalis* was documented only one case each. *Candida albicans* was found to statistically highly significant ($p<0.01$) when compared rest of the other organisms and also positively associated with younger age group between IQR 32-36 years. *C. albicans* is the most prevalent among all *Candida* spp. For the causation of oral candidiasis age group between IQR 32-45 years and it was found to be statistically significant ($P<0.01$).

KEYWORDS: Diabetics and Non-Diabetics, *Candida Albicans*, Spectrum, Incidence

INTRODUCTION

Back Ground

Diabetes mellitus is a common and growing global health problem which causes several complications. Periodontal diseases are considered the sixth complication of this disease. Diabetics have an increased predisposition to the manifestations of oral diseases like candidiasis, which is associated with poor glycemic control and therapeutic dentures [(1)]. This predisposition also contributes to xerostomia, which may be due to increased glucose levels in oral fluids or immune dysregulation.[2] Wearing complete denture is also known as another risk factor, which can promote colonization of *Candida*, produce Candidal biofilm and result in oral candidiasis. Association of denture and diabetes can increase the incidence of oral *Candida* disorders in diabetic patients. *Candida* species are present in the oral cavity of almost half of the population without causing disease.[2]

Asymptomatic carriage may cause a higher risk of *Candida* associated complications through yeast infections if they become immunosuppressed.[3,4] Colonization of Candida is more prevalent in people with diabetes mellitus[5–7] and many studies have shown a higher prevalence of *Candida* colonization in the oral cavity of diabetics compared with non-diabetic individuals.[1,8] Insignificant increase in frequency of oral candidal carriage and a significant increase in density of carriage was seen among diabetic patients[2]. However, they used a different sampling technique (imprint culture, i.e.

using swabs) [10] were Frequency of oral candidiasis in diabetics was increased but the density of candidal carriage was not found to be statistically significant. Strain diversity testing showed *Candida albicans* as the most prevalent species while non-albicans species were found to be increased in diabetics. They also demonstrated a lowered anti-fungal susceptibility in non-albicans isolates from diabetics[(1).] Oral candidal colonisation in DM, demonstrated that denture-wearing and oral symptoms were not related to oral candidiasis while smoking was a relevant risk factor. *Candida albicans* was the most isolated species in this study as well[(3)]. In Indian perspective very limited literature is available on incidence of Oral Candidal Colonisation in diabetes mellitus and identification of most prevalent species using Chromagar candida. In this context the present study aims to compare the frequency and density of oral candidal colonisation in patients with diabetes mellitus with that of non-diabetics, identify most prevalent Candida species colonizing the oral cavity in diabetics and non-diabetics using CHROMagar candida and to know the spectrum of infections by non-albicans species in diabetics with oral candidiasis.

MATERIALS AND METHODS

The observational control study conducted at Bangalore Medical College and Research Institute, Bangalore, India. Patients recruited with written consent and institutional ethical clearance was obtained for this study. All blood samples were collected between 9am and 12 noon to prevent circadian variations for diabetics evaluation. Detailed demographics history diabetic ; diabetes type, duration, therapy and presence of diabetes related systemic diseases and local factors like denture status, brushing routine and smoking habits were collected systematically with less profounded error and greater accuracy. All eligible patients meet their inclusion and exclusion criteria. Inclusion criteria**;** Diagnosis of diabetes mellitus (either type 1 or type 2) in patients of age 18yrs or older of both sexes, Age-matched healthy individuals without diabetes for control. Exclusion criteria**;** Patients with oral candidiasis who are already on treatment, Individuals who received antibiotics or steroid therapy or who had been using antiseptic mouthwash for prior three weeks. Patients with any other severe illness, especially HIV-AIDS. Asthmatics on corticosteroid bronchodilators. Subject of 50 individual control group was considered. The oral cavities of the subjects were examined for presence of signs of oral candidiasis like white coating or pseudomembrane, glossitis, stomatitis, etc. Irrespective of the presence of any signs and symptoms, samples were collected from each subject using an oral rinse technique described by Samaranayake et al.15. Each subject participating in the study was given a sterile wide-mouthed capped container containing 10ml of sterile phosphate buffered saline (PBS). The subjects were then asked to swirl the 10ml of PBS around the mouth for 60 seconds thoroughly and expel the mouth rinse into the sterile container. The samples were immediately sent to microbiology laboratory. Following concentration by centrifugation, using a sterile inoculating wire of volume 0.01ml primary isolation was done on Sabourauds Dextrose agar at 37^0C for 24-48 hours to check for growth of Candida and then subcultured onto CHROMagar Candida ™ at 37^0C for 24-48 hours for species identification. 16, 17. Colony forming units (CFU/ml) were calculated using the formula: CFU/ml = Number of colonies x 100. Collected data was analysed by using MINITAB-10.50 version, Univaraiate and multivariate analysis was used to draw the significant inference.

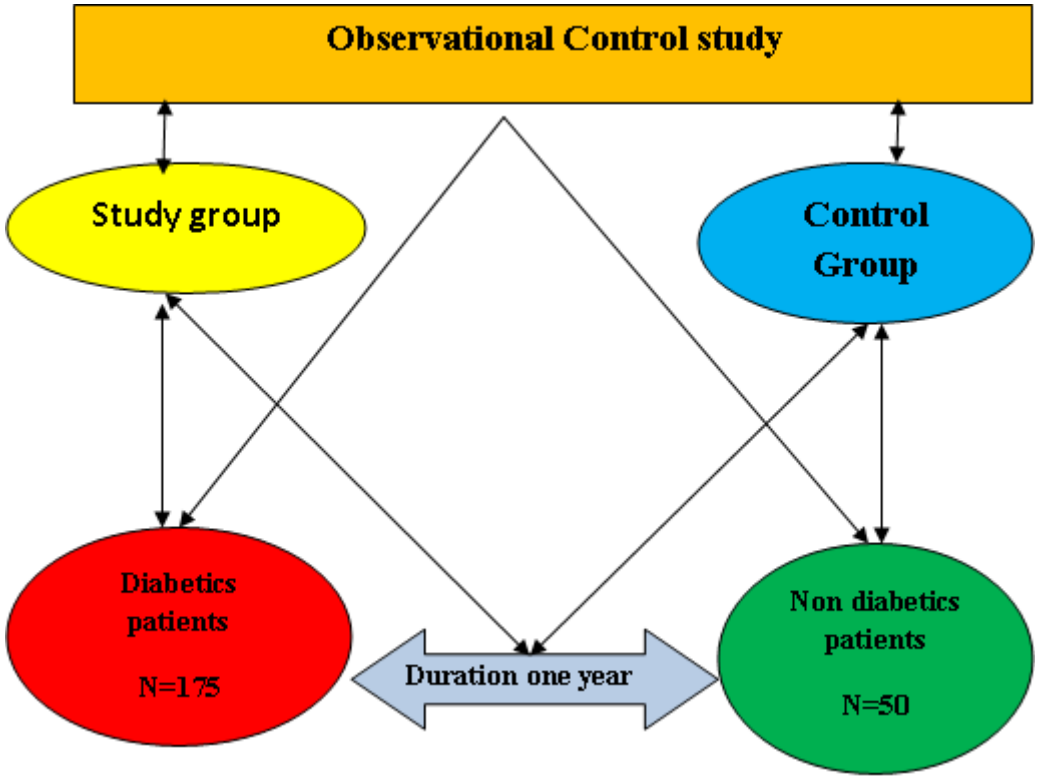

Figure 1: Scematic Diagram of Study Design

RESULTS

Table 1: Multivariate Analysis and Relation between Different Categorical Variables of Study and Control Groups

SL	Variables	Mean ±SD	IQR	CI-95%	P-value
I	Age(Years)	54.75±12.82	50.02-58.36	29.62-79.87	0.002**
01	Male	56.48±10.34 (68)	49.68-58.99	36.20-76.77	0.032*
	Female	53.64±14.09 (107)	51.23-55.86	26.11-81.27	0.012*
II	**Type of Diabetics**				
	Type -I	19- (10.85%)	18.32-20.19	16.32-23.03	0.221ns
	Type-II	312-(89.41%)	308.02-318.30	309.09-321.26	0.023ns
III	**Treatment**				
	Insulin	05-(2.85%)	2.66-6.21	1.96-6.89	0.632ns
	Insulin+OHG	13-(7.42%)	10.12-14.25	11.02-16.98	0.562ns
	OHG (Oral Hypoglycaemic drugs)	118-(67.42%)	113-10-120.36	109.21-126.36	0.023ns
	Not on Therapy	36-(22.28%)	32.47-39.62	33.02-38.62	0.052ns
IV	**Oral symptoms**				
	AS (angular stomatitis)	02-(1.14%)	1.52-3.21	0.62-2.58	0.85ns
	CT (coating on tongue)	12-(6.85%)	10.26-13.58	9.63-15.47	0.049ns
	H (halithosis)	05-(2.85%)	3.62-6.23	2.01-6.48	0.63ns
	PH (poor hygiene)	02-(1.14%)	1.08-3.14	0.34-3.19	0.25ns
V	**Associated Complications**				
	Dia Foot ulcer	04-(2.28%)	1.14-5.62	1.39-5.84	0.87ns

	Dia Neuropathy	04-(2.28%)	3.02-6.47	0.98-5.02	0.82ns
	Dia Retinopathy	06-(3.42%)	2.62-7.11	3.02-8.63	0.76ns
	Gangrene	01-(0.57%)	----	----	---
	Nil	160-(91.42%)	158.30-165.58	151.26-167.63	0.023ns
VI	**B. Routine**				
	BD	25-(14.28%)	23.26-29.63	20.19-28.01	0.02*
	OD	136-(77.71%)	132.14-139.03	129-33-139.41	0.00**
VII	**Follow up Details**				
	FBS	154.79±70.96 (126)	145.22-168.36	14.91-293.87	0.263ns
	PPBS	238.40±97.43 (115	223-269.32	47.43-428.96	0.311ns
	Duration of Diabetics	4.33±5.11 (175)	2.58-6.74	-5.68-14.34	0.658ns
VIII	**Expressed Organisms**				
01	Candida albicans	55-(31.43%)	48.11-58.16	43.69-59.62	0.001*
02	Candida albicans + *Candida parapsilosis*	01-(0.57%)	---	---	-----
03	*Candida dubliniensis*	04-(2.29%)	1.16-5.63	2.02-6.32	0.545ns
04	*Candida guilliermondii*	02-(1.14%)	0.63-0.98	0.32-1.26	0.801ns
05	*Candida krusei*	03-(1.71%)	0.91-4.26	0.74-3.89	0.852ns
06	*Candida parapsilosis*	05-(2.86%)	1.02-6.38	1.33-6.70	0.263ns
07	*Candida tropicalis*	01-(0.57%)	---	----	---
	Total	**71-(40.57%)**	**67.44-73.62**	**65.03-75.63**	**0.012ns**

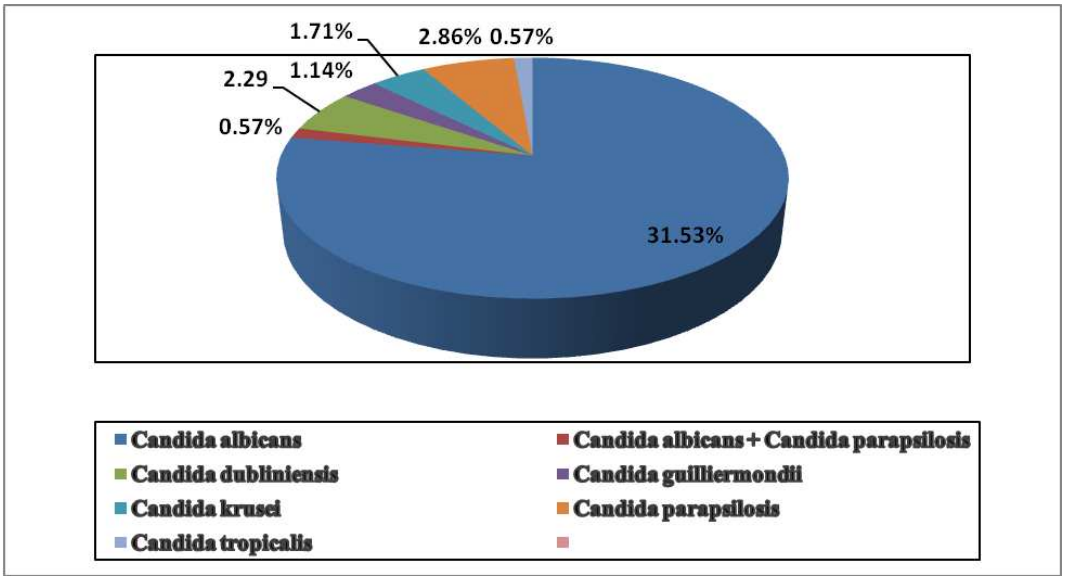

Figure 2: Distribution of Microbial Species

Table 2: Age Matched Relation between Candida Species

SL	Albican Species	Mean Age Mean±SD	CI-95%	Correlation Values	P-Value
01	Candida albicans	32.25±3.23	25.91-38.55	0.82	0.0*
02	Candida albicans + *Candida parapsilosis*	45.63	---	--	-
03	*Candida dubliniensis*	56±12.52	41.56-80.53	0.63	0.00*
04	*Candida guilliermondii*	62.11±15.45	31.82-92.32	0.45	0.22ns
05	*Candida krusei*	53.65±8.52	36.95-70.34	0.21	0.14ns
06	*Candida parapsilosis*	52.33±6.52	39.55-65.10	0.56	0.36ns
07	*Candida tropicalis*	65.00		--	

**, Significant at 1% level, ns-non significant

RESULTS

A total 175 patient's known type-I and type-II diabetic patients recruited with written consent .Of which male comprises 68 and female was 107 with mean age of male was 54.75 ±12.82 years IQR (50.02-58.36 years) and female mean age was 56.48±10.34 years IQR ;49.68-58.99 years. As per the analysis age was showed to be statistically significant ($p<0.05$) with incidence of Candida for both groups. The concurrent matrix of study design the incumbent group of type I and type-II diabetics was distributed 19(10.85%) and 312 (89.41%) respectively with inferred confidence interval 16.32-23.03 and 309.09-321.26. Group I is found to be statistically non significant when compared with group I ($P>0.05$). Total 131 patients were taking Insulin and OHG therapy; Of which Insulin 05 (2.85%) CI -95% 1.96-6.89; Insulin with OHG was 13 (7.42%), CI -95% 11.02-16.98 ;OHG was 118 (67.42%) ,CI 95% 109.21-126.36 and patients not on therapy was 36(22.28%) with defined CI -95% 33.02-38.62. The patient's treatment on OHG and not on therapy was statistically significant when compared to insulin and with combination of OHG. The associated symptoms was correlate with therapeutic parameters, present study documented Angular stomatitis 02(1.14%) CI-95% 0.62-2.58, Coating on tongue 12 (6.85%) CI-95% 9.63-15.47; Halithosis was 05 (2.85%) and Poor hygiene 02 (1.14%); CI 95% 0.34-3.19. The oral symptoms was positively associated with incidence of coating of tongue as compared with others ($p<0.05$). Different organism were found during culture with good ambient condition, as per the result, the more incidence was found in *Candida albicans* 55 (31.43%) followed by *Candida parapsilosis* 05(2.86%), *Candida dubliniensis* - 04 (2.29%), *Candida krusei* 3(1.71%). *Candida albicans +Candida parapsilosis* and Candida *tropicalis* was documented only one case each. *Candida albicans* was found to statistically highly significant ($p<0.05$) when compared rest of the other organisms and also positively associated with younger age group between IQR 32-36 years. Follow up visit has recorded with greater accuracy and lesser error at the level of 99.0%, the result found that the mean duration of diabetics was 4.33±5.11 years with confidence CI 95% -5.68-14.34 with mean FBS was 154.79±70.96 micro/dl and PPBS was 238.40 ± 97. 43 micro/Dl.

DISCUSSIONS

Clinical evidence of oral candidal infection was seen in 11 (8.3%) diabetic patients, 4 of which were overnight denture wearers and tobacco smokers. None of the controls had any clinical evidence of oral *Candida* infections. A statistically significant difference ($P< 0.001$) was detected 4 between diabetics and healthy controls in terms of positive yeast culture in that 77 diabetic subjects(58.3%) showed positive yeast compared with 39 (30%) of the healthy controls. As shown in table 2, *C. albicans* was the most prevalent species in both diabetics (81.8%) and controls (76.9%), followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. Of interest was the isolation of 1.3% of *C. kefyr* and *C. krusei* only from diabetics (Table 2). As shown in figure 1, *Candida* was detected more frequently in diabetic denture wearers patients than

in the control denture wearers in all sites which were sampled ($p<0.05$). Floor of the mouth, anterior palate and posterior tongue were the most frequently colonized oral sites. Figure 2 shows that the prevalence of *Candida* was significantly higher in diabetics both in denture wearers and dentate patients compared to healthy controls ($p<0.05$). The frequency of *Candida* isolation was significantly higher in smokers than in the non-smokers both in diabetic and controls ($p< 0.001$). Diabetes is rapidly becoming a major public health problem worldwide 12. The prevalence of oral *Candida* infections among Jordanian patients with diabetes mellitus in the current study is consistent with numerous previous studies, which have shown that diabetes mellitus is a major predisposing factor to symptomatic candidosis, oral or otherwise 5-7,18. This is also in agreement with numerous previous studies, which have all indicated that diabetes mellitus enhances *Candida* colonization and proliferation 6,7,19-21. Tapper-Jones *et al*. have shown that 42% of healthy nondiabeticsharbor *C. albicans* in their mouths compared to 60% of diabetics6. Yarahmadi *et al* have suggested that 16.2% of the controls and 40.2% of the diabetics carry *C. albicans* in the mouth 22. The threshold of sensitivity has been found to be lower for buccal swabbing than for imprint or saliva collection, therefore, the overall percentage of individuals carrying yeast isolates in the oral cavity may be slightly lower than what is reported in the present study20. The mechanisms by which this could occur are numerous, for example, the induction of immune incompetence, the availability of increased levels of sugar in the oral microenvironment and the method used to obtain samples and the site sampled within the oral cavity. In addition to diabetes mellitus, the prevalence of oral *Candida* infections is influenced primarily by smoking 7, 23-25. It is also clear from the findings presented in this study that dentate individuals whether diabetics or not are less prone to *C. albicans* colonization. Despite several previous studies, which have looked into this issue more closely, the exact mechanism by which this significant difference between dentate and denture wearers cannot be readily explained 23-25. It is generally assumed that elderly individuals have higher yeast carriage rate, although, this may be more due to denture wear and increased medication than due to changes in host physiology.

CONCLUSIONS

It is clear that diabetics are more susceptible to oral candidiasis than non-diabetics. Furthermore, smoking and denture wears are at high risk of being infected. *C. albicans* is by for the most prevalent among all *Candida* spp. as the cause of oral candidiasis.

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