The prevalence of *Candida spp.* in the saliva of controlled and uncontrolled diabetes mellitus type II patients

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ABSTRACT

Background: Diabetes mellitus type 2 has been known for many years as the most common endocrine metabolic disorder that affect the oral cavity and cause many oral diseases including candidiasis. In this study, the incidence of *Candida spp.* in the saliva of controlled and uncontrolled diabetic patients were determined and compared with non diabetic group.

Material and method: The sample consists of 200 subjects: 100 diabetic patients [57 (28.5%) uncontrolled diabetes, 43 (21.5%) controlled diabetes] and 100 (50%) non diabetic groups. Saliva samples was obtained from the subjects and cultured on selective media using appropriate microbiological method to observe the presence of *Candida spp*.

Results: The results revealed a significant association ($p < 0.001$) between diabetic patients and the presence of *Candida spp.* using statistical analysis. The odds ratio of the presence of *Candida spp.* in the controlled and uncontrolled diabetic patients were 0.539 (95% CI= 0.193, 1.508).The odds ratio of the presence of *Candida spp*. in the uncontrolled and controlled diabetic patients were 17.433 (95% CI= 7.298, 41.642) and 9.40 (95% CI = 4.068, 21.686), respectively, compared to non diabetic group. A significant association was found between the Presence of *Candida spp.* and the following variables: Groups ($p < 0.000$), Gender ($p < 0.000$), Smoking ($p < 0.000$), Antibiotics ($p < 0.000$), oral mouthwash ($p < 0.000$) Edentulous ($p < 0.000$) and Denture wearing ($p < 0.000$).

Conclusion: *Candida spp*. population significantly increased in the oral flora of diabetic patients compared with non diabetic group.

Keywords: Diabetes mellitus type II, Controlled and Uncontrolled diabetes, *Candida spp.* (J Bagh Coll Dentistry 2013; 25(4):171-176).

INTRODUCTION

Diabetes mellitus (DM) has been known for many years as the most common endocrine metabolic disorder and its occurrence increased worldwide, it affects about 14 million people in the United States, the number of new cases increases by more than 700, 000 per year [(1)]. The first broadly accepted classification of diabetes mellitus was published by World Health Organization in 1980 [(2)].

The expert committee proposed in 1980 two main modules of diabetes mellitus and named them, insulin dependent diabetes mellitus (IDDM) or Type 1, and non insulin dependent diabetes mellitus (NIDDM) or Type 2 [(2)]. Basically, this study will be focused only on Type 2 DM because patients with this type of DM are suffering from many complications such as neuropathy, nephropathy, blurring vision and oral complications.

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Type II DM can be classified based on the degree of the disease into controlled diabetes and uncontrolled diabetes based on glycosylated hemoglobin (HbA1c) serum levels [(3-5)].

From all the best known systemic diseases, diabetes has been the most frequently blamed as a risk factor for oral pathogenic disorders such as candidiosis [(6)]. The saliva contains a great number of microorganisms (approximately 10^8 per ml). Most of the microorganisms in the saliva are derived from other parts of the oral cavity such as the teeth and oral mucosal surfaces as a result of mechanical abrasion caused by chewing, talking and swallowing. The microvascular changes and possibly increased glucose concentration in the saliva and gingival crevicular fluid which might contribute in declining pH of saliva resulting in acidogenic microorganism substrate and plaque formation. As a Result of that, the increased growth of acidogenic microorganisms such as *Candida albicans* will had a prominent role in developing various oral complications [(7)].

Based on some studies indicated that the degree of metabolic control may play an important role in the oral manifestations of diabetes whether the type of disease is insulin dependent diabetes mellitus (IDDM) or non insulin dependent diabetes mellitus (NIDDM). The uncontrolled DM may cause tissue

destructions leading to advanced periodontal and oral fungal infections.

Although there are many published reports on the high presence of *Candida spp.* in the oral cavity of diabetic patients than in non-diabetic subjects [8-10], studies regarding the presence of these species in a specific criteria of type 2 diabetes are still unknown. Therefore, the aim of this study is to determine the presence of *Candida spp.* in the controlled and uncontrolled diabetes mellitus type II patients and compare it with non diabetic groups.

SUBJECTS AND METHODS

Study groups

The data were collected from patients attending the diabetic clinic at Hospital University Sains Malaysia (HUSM) in Kota Bahru, Malaysia during a period of two months started from 9th January 2011 to 8th March 2011. Non-diabetic group subjects were selected after checking their fasting blood glucose from outpatient clinic in school of dental sciences at University Sains Malaysia (USM). After obtaining the permission from the patients to participate in this study by signing a consent form given to them, data were collected.

A total of 100 type 2 diabetic patients (39 males and 61 females) and 100 non diabetic groups (39 males and 61 females) were included in this study based on a sample size formula suggested by Daniel (1999) [11]. The controlled and uncontrolled type 2 diabetic patients were verified by checking the glycosylated hemoglobin (HbA_{1c}) from patient records in the diabetic clinic after taking the permission from the clinic. Patients with an average age from 40 – 60 years having type 2 diabetes were included in this study. Severally debilitated subjects such as neuropathy and necrosis (gangrene) were excluded from this study after checking their records from the clinic.

Sample collection

Swabs were taken from the floor of the mouth of 200 individuals included in the study. The swabs were placed in sterile transport media to maintain the validity of the microorganisms, and then they were transported to the oral microbiology lab at the school of dental science for processing.

Procedure of culturing and isolating *Candida Spp.*

The samples were cultured immediately on Sabouraud Dextrose Agar (SDA) using streaking method described by (Pollack *et al*., 2002) [12] and incubated aerobically for 48h at 37ºC. After incubation, well isolated colonies were picked up for identification purposes and transferred into a sterile nutrient broth and incubated at 48h at 37ºC. Each culture was checked for purity by subculturing it onto another SDA plate by transferring 0.1 ml of the sample on to the plate and spread the entire plate using a sterile hokey stick and incubated with the same circumstances. Most of the species that have been identified were *C. albicans*. The growth of *Candida* was identified by smooth, creamy white dots (Figure 1. and Figure 2.).

Statistical analysis

SPSS version 14.0 was used for data entry and analysis. The association between the study groups and the presence of *Candida spp.* were compared using chi-square test. Univariate logistic regression was used to determine the association between the presences of *Candida spp.* with groups, gender, smoking, alcoholic, antibiotics, oral mouthwash, edentulous and denture wearing. Covariates such as age, height and weight are reported as mean ± SD. $P \leq 0.05$ was considered statistically significant.

Figure 1. The growth of *Candida spp.* on Sabouraud dextrose agar

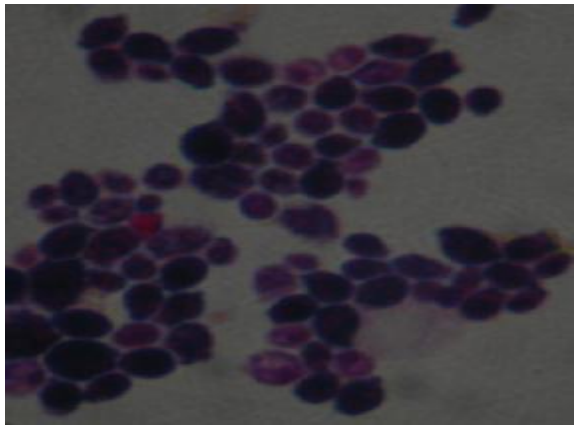

Figure 2. Gram positive round *Candida* cells

RESULTS

Demographic characteristics

In those present study, the majority of diabetic patients were Malay (86%) as it is the predominant race in Kelantan state were the diabetic disease has been reported to be higher at 10.5% as contrast to 8.2% for the rest of Malaysia [13]. The distribution of demographic characteristics between the groups is shown in Table 1.

Table 1. Demographic characteristics between diabetic and non diabetic groups

Variables	Diabetic group	Non diabetic group
	n (%)	n (%)
Gender		
Male	39 (19.5)	39 (19.5)
Female	61 (30.5)	61 (30.5)
Race		
Malay	92 (46)	80 (40)
Chinese	7 (3.5)	8 (4)
Indian	1 (0.5)	10 (5)
Others	0 (0)	2 (1)
Educational level		
< Primary	36 (18)	8 (4)
Secondary	46 (23)	52 (26)
Tertiary	18 (9)	40 (20)
Occupation		
Employee	36 (18)	67 (33.5)
Housewife	53 (26.5)	30 (15)
Retired	11 (5.5)	3 (1.5)
Age (mean ± SD)	58.6 ± 8.7	39.4 ± 14.8

Note. SD= Standard Deviation, n= number of participants

Description of diabetic patients

Most of the subjects were suffering from diabetes less than ten years. All diabetic patients were taking medications except 2 (2%) patients didn't take any medications. Of the 100 diabetic patients, 82 (82%) were taking other medications besides medicine for diabetes such as anti HPT, cholesterol and other medications, The description of diabetic patients is shown in Table 2.

Table 2. Description of diabetic variables among diabetic patients

Variables	Controlled diabetes	Uncontrolled diabetes
	n (%)	n (%)
Diabetes duration		
< 10 years	28 (28)	30 (30)
10 years	3 (3)	7 (7)
> 10 years	12 (12)	20 (20)
Diabetic pills	43 (43)	55 (55)
Other medications that diabetic patients take	33 (33)	49 (49)

Note. n= number of participants

Associations of *Candida spp.* and study groups

It was observed that older men patients show significantly higher incidence of *Candida spp.* in the oral flora ($P < 0.000$). Most of the previous studies [14-16] revealed that females are more vulnerable to oral *Candida* than males due to the hormonal factor and the great incidence of iron deficiency that might be responsible for the activity of the yeasts and thus leads to oral lesions [17]. However, there is no social or physiological characteristic that could influence the incidence of oral *Candida* among men except for some habits such as smoking that could be the main factor for the presence of some yeast that mainly occurs among men.

The prevalence of *Candida spp.* among diabetic patients was higher than that in the non diabetic group. Diabetic patients are more vulnerable to oral *Candida* infections than non diabetic groups due to factors that promote oral fungal infections accredited to many reasons including hyperglycemia, xerostomia,

hyposalivation that reduce the salivary flow rate and oral habits. These findings are supported by many studies [8-10,18,19]. Oral candidiasis, which is a disease caused by the genus *Candida*, is an opportunistic fungal infection generally associated with hyperglycemia and is thus a common complication of marginally controlled or uncontrolled diabetes [20]. This statement is supporting the findings of this study that showed controlled and uncontrolled diabetic patients are more prone to oral candidal infections that caused by *Candida spp.* compared to non diabetic group. The results have shown that the odds for the prevalence of *Candida spp.* in the uncontrolled and controlled diabetic patients are 17.433 [95% CI= 7.298, 41.642] and 9.40 [95% CI= 4.068, 21.686] times more, respectively, compared to the non diabetic group. According to a previous study done by Hill *et al.* [21], who studied the association of predisposing factors related to diabetic patients such as glycosylated haemoglobin with oral *Candida*. They found that patients with glycosylated haemoglobin above 12% (considered as uncontrolled diabetic) were strongly associated with oral fungal infection. The odds ratio of Hill *et al.* [21] was 13.00 times more than non diabetic group. The results of this study showed a higher incidence of *Candida spp.* in the uncontrolled diabetic with odds ratio of 17.433 times more compared to non diabetic groups. Patients who were uncontrolled diabetes may suffer from many complications including increased glucose level in saliva, decreased saliva secretion, delayed healing and other pathological changes which can contribute to high susceptibility of the oral tissues to infection and local irritants [22]. However, the current study didn't found any significant difference in the prevalence of *Candida spp.* between specific criteria of type 2 DM because of the low data that have been collected

Smoking habit was found to be an important risk factor for prevalence of *Candida spp*. among the subjects. The results of this study have shown a significant association between smoking habit and *Candida spp*. Smoking has been known as an important factor in the oral candidal infection [23]. This finding is in agreement with Khaled *et al.* [18] who also found a significant association between smoking and the presence of *Candida spp*. ($p < 0.001$). Although, the percentage of smokers among the subjects was low compared to non smokers who were significantly high but it still considered as a risk factor for oral candidal infection.

Alcoholism is another oral habit which can lead to a variety of oral lesions that caused by *Candida spp.* [24]. However, no significant association was found between alcoholism and *Candida spp.* in this study because of the low percentage of alcoholic subjects. Therefore, there is a less possibility of considered it a risk factor in this study. The results are also shown a significant association between oral mouthwash and *Candida spp.* ($P < 0.000$).

The number of subjects who were using oral mouthwash or oral rinses were 60 (30%) compared to 48 (24%) of whose who were not using these solutions. Although, the presence of *Candida spp*. was less among subjects who were not using mouthwashes in this study, they are still at risk of having oral Candidal infections.

It is known from the results of this study that there is a significant association between edentulous and the prevalence of *Candida spp*. ($p < 0.000$). Subjects who were edentulous showed a high percentage of *Candia spp*. 72 (36%) in their oral cavity compared to those who were not edentulous 36 (18%). According to Price *et al*., 1982, common oral complications are associated with diabetes include tooth loss, gingivitis, periodontitis and pathological changes of oral soft tissues. This statement is in agreement with the results of this study.

Patients who wear dentures in their oral cavity, associated with the limited changes of the oral mucosa and the systemic complications, are more susceptible to *Candida* infections [15]. A significantly higher incidence of *Candida* infection and increased levels of *Candida spp.* were found in diabetic patients wearing removable dentures [6,21, 25]. These findings are in agreement with the results of this study that shows a significant association between *Candida spp.* Several complications may occurs in patients who wears removable denture such as the decrease of salivary pH and saliva flow rate that slow down the mechanical cleaning process of the soft tissue surfaces by the tongue and thus, it reduced the tissue resistance against infections [26]. Therefore, diabetic patients wearing removable denture in this study are at high risk of having candidal infections in their oral cavity.

There was a significant association between the prevalence of *Candida spp.* and antibiotics ($P < 0.000$) in this study. The results showed the prevalence of *Candida spp.* was higher among subjects who were non taking antibiotics 91 (45.5%) compared to those who were taking antibiotics 17 (8.5%). The ignorance of taking the appropriate antibiotics including antifungal will increase the prevalence of *Candida spp.* and thus, it will lead to oral fungal complications.

According to Paula *et al.* [16], the knowledge of antifungal susceptibility testing and the

development of new antifungal drugs are compulsory to accomplish a decrease in *Candida* infections and raise the quality of life denture-wearing individuals with and without type 2 diabetes mellitus. The association between the presence of *Candida spp.* and variables is shown in Table 3.

As conclusion; the presence of *Candida spp.* is more frequent among diabetic patients than non diabetic patients. Diabetic patients showed a higher incidence of *Candida spp.* than non diabetic group. Generally, diabetic patients have a low resistance to infections including oral candidal infections due to suppressed (low) immunity. Factors such as smoking, edentulous, and denture wearing are the main factors for the prevalence of *Candida spp.* The refusal to take antibiotics or use oral mouthwashes as recommended by dentists also increases the risk of oral *Candida* infections. Further studies on the prevalence of these species among specific criteria of type 2 DM is recommended.

Table 3: The association between the presence of *Candida spp.* and other variables

Variables	Presence of *Candida spp.*		Crude OR	95% CI	*P* value
	Yes	No			
	n (%)	n (%)			
Group (1)					
Diabetic	82 (41)	18 (9)	12.97	[6.581, 25.546]	0.00
Non diabetic	26 (13)	74 (37)			
Group (2)					
Controlled DM	33 (16.5)	10 (5)	9.40	[4.068, 21.686]	0.00
Non diabetic	26 (13)	74 (37)			
Group (3)					
Uncontrolled DM	49 (24.5)	8 (4)	17.433	[7.298, 41.642]	0.00
Non diabetic	26 (13)	74 (37)			
Group (4)					
Uncontrolled DM	49 (24.5)	8 (4)	0.539	[0.193, 1.508]	0.17
Controlled DM	33 (16.5)	10 (5)			
Gender					
Male	64 (32)	13 (6.5)	8.84	[4.385, 17.818]	0.00
Female	44 (22)	79 (39.5)			
Smoking					
Yes	28 (14)	0 (0)	2.150	[1.832, 2.524]	0.00
No	80 (40)	92 (46)			
Alcohol consumption					
Yes	2 (1)	0 (0)	1.87	[1.641, 2.127]	0.19
No	106 (53)	92 (46)			
On antibiotics					
Yes	17 (8.5)	0 (0)	2.011	[1.738, 2.326]	0.00
No	91 (45.5)	92 (46)			
Oral mouthwash					
Yes	60 (30)	24 (12)	3.542	[1.943, 6.457]	0.00
No	48 (24)	68 (34)			
Edentulous					
Yes	72 (36)	0 (0)	3.556	[2.695, 4.690]	0.00
No	36 (18)	92 (46)			
Denture wearing					
Yes	65 (32.5)	0 (0)	3.140	[2.453, 4.014]	0.00
No	43 (21.5)	92 (46)			

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