



**ASSESSMENTS FREQUENCY OF ORAL FUNGAL INFECTION WITH TYPE 2 DIABETES
MELLITUS IN IRAQI PATIENTS**

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Abstract

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder with a vital genetic component. Recently, there was a positive association between the prevalence of oral fungal infection and disease. The current study aimed to assess the frequency and prevalence of oral fungal infection in T2DM in Iraqi patients. Methods: seventy-five patient swabs were collected from oral and same numbers from the healthy person as control with age range (40-46 years) and different genders. The routine methods and advanced techniques such as the Vitek 2 system were used to identify organism species. Result: Our findings indicated that *C.albicans* were more frequently associated with T2DM patients than healthy controls, with 32 (42.7%) of T2DM patients infected vs 12(16%) healthy controls. There is a clear role of glucose concentration in serum patients with the distribution of fungi and their impact on them. *C. albicans* recovered from 32 out of 46 patients, with mean glucose concentration (192.7 ± 28.6), followed by *C.krusei* and *C.ciferrii*, isolated from five patients with mean glucose concentrations of (216.3 ± 7.1 and 221), respectively. Conclusion: *C. albicans*, *C.krusei* and *C.ciferrii* were high frequency with T2DM and increased in patients with a high mean concentration of glucose.

Keywords: *Candida albicans*, Glucose concentration, and T2DM.

Introduction

Diabetes is one of the most perplexing health conditions of the twenty-first century. Type 2 Diabetes Mellitus (T2DM) accounts for 90–95 % of all diabetes cases, ranging from insulin resistance with relative insulin deficiency to insulin secretory defect. Diabetes is one of the most perplexing health conditions of the twenty-first century. Type 2 Diabetes Mellitus (T2DM) accounts for 90–95 % of all



Diabetes cases were range from insulin resistance with relative insulin deficiency to insulin secretory defect (Punthakee et al ., 2018). The abnormal insulin secretion or the impairment in insulin action could affect blood sugar and its deficient action on target tissues and cause DM (Leete et al .,2020; Verhulst et al., 2019). Classification of diabetes mellitus (DM). DM is divided into two main categories: T1DM, caused by an absolute insulin deficit, and T2DM, caused by IR and an insufficient compensative insulin increased production. Epidemiology of T2DM, In developing countries, 87–91% of people with DM have T2DM, 7–12% have T1DM, 1–3% have other DM and show that 425 million people with DM worldwide were aged 20–79, and half (49.7%) people who live with DM have not been diagnosed, with 352 million people living with impaired glucose tolerance (IGT), 7.3 % of 20-79 years old adults in The urban compared with the rural environment (10.2 vs. 6.9%) is more familiar with DM(Awad et al.,2021). Hyperglycemia occurs when the pancreas produces insufficient insulin, insulin defect, or both (Spindler et al ., 2016). Typically, the human body employs unique defence systems to prevent bacteria, viruses, fungi, poisons, and parasites from invading its body. These pathogens are difficult to infiltrate this defensive mechanism under normal circumstances. Nevertheless, various conditions and disorders impair the immune system's function. For instance, bacteria may readily enter and cause disease (Tessarò et al .,2017; Lao et al.,2020). Unfortunately, diabetes impairs the immune response of the host. Additionally to the hazard of natural barrier degeneration induced by neuropathy, T2DM may impair cellular immunity (McNabney & Henagan.,2017). Infections are a significant concern for people with diabetes, according to the American Diabetes Association, since their immune systems are unable to fight off invading pathogenic microbes.

Methods

A case-control study was conducted From October to December 2020; 150 specimens were collected from the Endocrinology and Diabetes Center in Baghdad, including oral swabs and whole blood. Oral swabs were collected via AIMS transport media. Isolation and Identification of fungal isolates, the oral cavity is usable, including a smear via swab. Each smear collected from patients and healthy from oral cavity Microscopes, germ tube, growth at 45C, and Vitek 2 Compact system can be used for identification and differentiating hyphae, yeast forms, shape, and arrangement.

Statistical Analysis

The data were evaluated statistically using IBM SPSS Statistics 26.0. (Armonk, NY: IBM Corp). The scales data were subjected to a normality test, and other variables were tested in chi-square (X^2). Alpha type one error was **at ≤ 0.05** . A significant difference was utilized to compare categorical variables.

Results

1. Distribution of participants according to Age and Gender

Seventy-five T2DM patients were included in the present study, and 75 healthy control with approximately the same age range and gender were used as healthy control. Table (1) shows no significant difference between them, whereas the mean \pm standard deviation of patients aged was



countered (40.2±13.4)vs. (43.1±13.8) healthy controls, as for females, in the patient were countered(43.1±14.7) vs (46.0±13.2) healthy control. The distribution of groups according to gender was shown in Figure (2) and revealed that female patients were somewhat more prevalent than males in this study. There were represented 43 (57.3 %) and 32(42.7 %) respectively .while in healthy control males were more prevent than females with a percentage 42(56%), 33(44%) respectively. According to Chi-square analysis (X^2), there was no statistically significant difference between the gender of patients ($X^2=1.6$, $p=0.204$).

Table (1): Distribution of patients and healthy control according to their age

Groups	Healthy Mean±SD	Patients Mean±SD	P-value
Male	43.1±13.8	40.2±13.4	0.374
Female	46.0 ± 13.2	43.1 ± 14.7	0.368

The mean significant difference is at ≤ 0.05 . It is highlighted in bold font. Statistically analysis by independent T-test.

Phenotypic Characteristics

The current study included 150 oral swabs collection. Seventy-five swabs were collected from T2DM patients who reported 58 (77.3%) positive oral fungal infections and 17(22.7%) negative infections. An interview with healthy controls Who reported 14(18.7) out of 75 was positive oral fungal infected, and 61(81.3) out of 75 were negatively infected, as shown in figure (1).

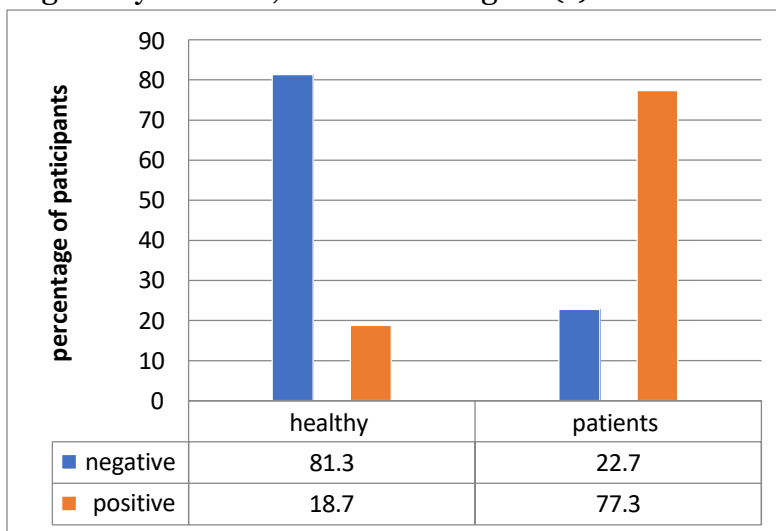


Figure (1): positive and negative oral fungal oral infection in T2DM patients and healthy control.



Figure (2) presented the distribution of oral fungal infection in the T2DM patients and healthy control. Our findings indicated that *C.albicans* was more frequently associated with T2DM patients than healthy controls, with 32 (42.7%) of T2DM patients infected vs 12(16%) of healthy controls, followed by *C.dubliniensis*, which was the second relative positive in patients 6 (8%) vs 1(1.3%) healthy controls, respectively. Moreover, *C.krusei* was associated with patients 4 (5.3%). On the other hand, other microbes have been isolated from patients.

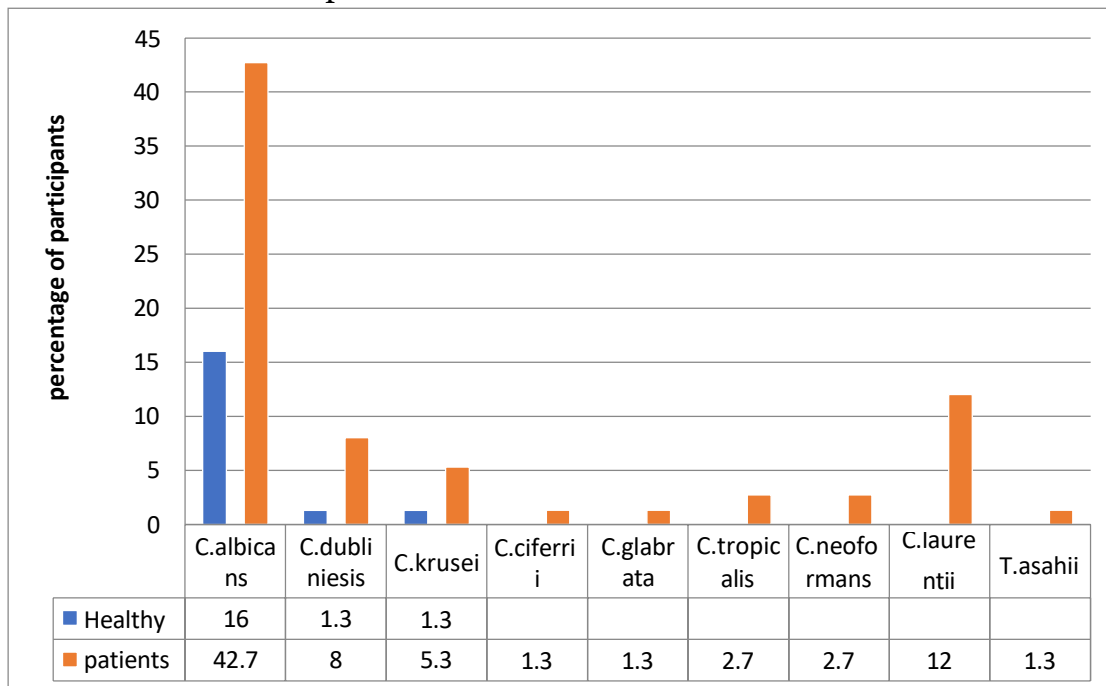


Figure (2): Distribution of oral fungal infected according to categories (T2DM patients and healthy control)

Distribution of Oral Fungal Infection According to Gender

The present study included 75 patients (32 males and 43 females) and healthy control subjects (42 males and 33 females). 24 (75%) of 32 males were infected with an oral fungal infection, while 8 (25%) of 32 patients were negative. 10 (23.8%) of 42 males were infected, while 32 (76.2%) were negative. As shown in (table 2). As for females, from 43 female patients were 34 (79.1%) were infected with fungal infection. Compared 9(20.9%) were not infected. Compared with females, healthy control was 4 (12.1%) out of 33 infected. At the same time, 29(87.9%) were negative. Statistically, the T2DM female patients were positively infected more than males.



Table (2): Distributed oral fungal infection (positive and negative) according to genders.

Gender	Males				Females			
	Patients	Healthy	X ²	p-value	Patients	Healthy	X ²	p-value
Positive	24(75%)	10(23.8)	19.1	<0.001	34(79.1%)	4(12.1%)	33	<0.001
Negative	8(25%)	32(76.2)			9(20.9)	29(87.9%)		
Total	32	42			43	33		
X ²	26	61.8			47.9	18.9		
p-value	<0.001	<0.001			<0.001	<0.001		

The Significant difference is at ≤ 0.05 level. It is highlighted in bold font—statistically analysis by X²: chi-squared test.

Role of High Concentration of Glucose With The Prevalence of Fungi

Table (3) presented a clear role of glucose concentration in serum patients with the distribution of fungi and their impact on them. *C. albicans* recovered from 32 out of 46 patients, with mean glucose concentration (192.7 ± 28.6), followed by *C.krusei* and *C.ciferrii*, isolated from five patients with mean glucose concentrations of (216.3 ± 7.1 and 221) respectively. *C.dubliniensis* was then isolated from six individuals with a mean glucose concentration of 196.6 ± 29.4 . Additionally, this investigation identified *C.neoformans* and *C.laurentii* with elevated glucose concentrations (230 ± 14.0 and 190 ± 23.4), isolating *C. neoformans* from two and *C. laurentii* from nine T2DM patients.

Table (3): Stratification of fungal types in T2DM patients depended on glucose concentration.

Fungal types	Total no.patients	Mean \pm SD glucose (ng/ml)
<i>Candida albicans</i>	32	192.7 \pm 28.6
<i>Candida dublieinsis</i>	6	196.6 \pm 29.4
<i>Candida krusei</i>	4	216.3 \pm 7.1
<i>Candida ciferrii</i>	1	221
<i>Candida glabrata</i>	1	160
<i>Candida tropicals</i>	2	190 \pm 42.4
<i>Cryptococcus neoformans</i>	2	230 \pm 14
<i>cryptococcus laurentii</i>	9	190 \pm 23.4
<i>Trichosporon spp</i>	1	152
Negative	17	168.5 \pm 33.6

SD: standard deviation, statistically analysis by frequencies with split file depended on microbial groups.



Discussion

The current investigation indicated that T2DM females marginally increased prevalence than males, aged 43.1 ± 14.7 , which might be explained by a genetic propensity to develop the disease and its combination with specific environmental or lifestyle variables. Additionally, that may be the interpretation of the role of age and gender in developing the disease. T2DM infected the mature adulthood is more than young, opposite T1DM. Our study agreed with the study done by Zimny et al. (2020) that also suggested that females increased more than males and were more suffering from the disease. The development and progression of T2DM have been subjected to some risk factors related to patients' age, gender, immunological status, and genetic background (Taha et al., 2018).

Previous studies have shown a high incidence (77%) of isolation of *Candida* spp. from the oral cavities of T2DM patients. A statistically significant difference in the number of patients and healthy control (Manfredi et al., 2019) agreed with our result. A high percentage isolated from patients was *C. albicans*. A similar study conducted by Tsang et al. (2017) was mentioned that The oral carriage of *C. albicans* in the test group ($n=76$; 36.2 %) was significantly greater than in the healthy control ($n=50$; 23.8%). Other *Candida* spp isolated included *C. tropicalis* (five isolates from DM and two from healthy control groups), *C. glabrata* (five isolates from DM and three from healthy control groups), *C. parapsilosis* (three isolates from DM and two from healthy control groups), *C. guilliermondii* (one isolate from the healthy control group) and *C. famata* (one isolate from the DM group). Another study was documented and focused on the more etiological effect of microbial associated with patients. The overall prevalence of *Candida* spp in DM with periodontitis observed in the study was 52%. The most common species of *Candida* identified were *C. albicans* (38%), followed by *C. dubliniensis* (9.5%), *C. tropicalis* (4.7%), and *C. glabrata* (4.7%) (Madathil et al., 2020). In Iran. The study indicated that *C. albicans* was more common in T2DM patients, had more pathogenicity effects, and may cause oral disease. (Noori et al., 2017).

Moreover, *Cryptococcus neoformans* and *C. laurentii* were associated with the patients who only accounted for 2 (2.7%), and *C. laurentii* was reported in patient 9 (12.0%). Furthermore, *Trichosporon asahii* was associated with one from the patient only 1 (1.3%). Our result agreed with Morales-López & Garcia-Effron. (2021) that indicated found yeasts in 74.8% of the patients. The difference from *Candida* spp was isolated 16.8% corresponded to *Rhodotorula*, *Trichosporon*, *Saccharomyces*, *Cryptococcus*, *Kloeckera*, and the *Prototheca* algae.

On the contrary, Diwan et al. (2018) observed that males (51.4%) surpassed females (39.6%) in the patient group, and there was no significant difference in their ages ($p=0.09$). It has been observed that the mature adulthood males and females gender are more susceptible to disease. A comparable investigation showed that females were more susceptible to developing T2DM than males.

This study observed fungal diversity in the T2DM patients at high frequency and significantly different from a healthy control. Furthermore, T2DM was more sensitive to fungal infections due to changes in the oral environment and changed with increased concentration of glucose and increased ketone body and same genetics diversity. Our result agreed with Rasoulpoor et al. (2021) indicated that more than 50 % form oral specimens were infected with peritonitis compared with healthy control. Further study



by Babatzia et al.(2020) observed that The overall prevalence of *Candida* in DM patients with periodontitis was 52% of a sample of the study.

The current study considers the first study to isolate *Cryptococcus* spp and *Trichosporon asahii* from oral T2DM patients in Iraq and suggested oral cryptococcal infections rarely have, but none, as yet. T2DM patients were immunocompromised, which gave a chance to oral infection. that agreement with Santosh et al . (2021) that oral fungal infections are most commonly due to *Candida* spp. Other fungal pathogens, oral and mucocutaneous cryptococcal infections, have been described seldom. *Candida* spp. is the most often seen fungal species in the oral cavity as typical resident flora, being detected in 75% of healthy persons, followed by *Cladosporium* spp. (65%), *Aureobasidium* spp., *Saccharomyces* (50% for both), *Aspergillus* spp. (35%), *Fusarium* spp. (30%), and *Cryptococcus* spp. (20%) (AL-Janabi et al .,2019). *Cryptococcus* spp was isolated from various tissues and fluids, including cerebrospinal fluid, blood culture, tissue biopsy, and bronchoalveolar lavage fluid (Sousa et al .,2020). According to the presented results, the clear significant separation between patients and Healthy control was the large range of microbial species diversity and altered patient composition compared to healthy control with small microbial species.

The current study proposed that T2DM female patients are more susceptible to oral fungal infection than males. This difference in the distribution of oral fungal infection according to gender and increase in females depended on genetic predisposition and differences in metabolism between them. This study agreed Sampath et al . (2019) indicated that oral infection of *Candida* in the Sri Lankan group of diabetics was significantly higher than their healthy counterparts. *Candida* spp has a predilection for colonizing the human oral cavity, particularly in patients females with DM accounted 125 (60.8%) from 200 participants in the study. The further study mentions gender distribution was approximately equal, with a slight predominance of females more than males (Monea et al .,2017).

In contrast, several studies were shown an increased chance of infection for males more than females. A study done by Hu et al.(2019) inspired the association between male gender and Candidiasis. The diversity of fungal infections between two genders is due to physical labour or metabolism differences and genetic variation (Tamai et al .,2020).

The current study assumed increased frequency of fungal infection in patients with an increased concentration of glucose that may be the increase of glucose in blood results insulin resistance that increased the concentration in the fluid of body such as saliva, therefore, available suitable environments to convert the fungal from normal flora to pathogenic fungal with lower with immune response. Our study agreed with Several studies that indicated a Positive correlation between blood glucose in diabetics and candidal carriage, which is similar to our result (Mishra et al .,2019). Moreover, Tukey et al. (2017) show a significant positive correlation between random fasting plasma glucose and salivary glucose in normal and unhealthy controlled DM subjects. In addition, A similar study conducted by Nouraei et al . (2021) also showed positive yeast in 58.3% of diabetics compared with 30% in healthy controls.



DM enhances candida colonization and proliferation. In particular, oral candidiasis and Candida infections have also been frequently recognized in DM due to increased glucose in their oral fluids and immune dysfunction (Figueira et al .,2020).

Conclusion

The current study focuses on increasing the frequency of oral infection with T2DM patients, detecting *C. albicans* more common prevalence between them, and increased frequency with increased glucose concentration. Females patients were increased frequency with oral infection more than males in Iraqi patients.

Abbreviations

T2DM: type two diabetes mellitus; CI: Confidence interval; PD: periodontitis. RA: Rheumatoid Arthritis; SD: Standard deviation; DM: diabetes mellitus; Th :thymus helper; impaired glucose tolerance (IGT)

Knowledge

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Declarations

Ethics approval and consent to participate

The participants provided their written informed consent to be included in the study. The College of Science (Al-Mustansiriya University) obtained the approval of the Ethics Committees at the target hospitals to carry out the study.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.



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