Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Microbiology Research

Journal homepage: www.ijmronline.org

Original Research Article

Comparison of mucosal oral fungal colonization between diabetic patients and non-diabetic individuals in Babylon using molecular identification

Rajaa A. A. Al Anbagi[1*], Fakhir R.H. Alshuwaili[2], Amna S. Chijo[1], Ghafran W. Awad[1]

[1]Dept. of Medical Biotechnology, Al-Qasim Green University, College of Biotechnology, Babylon, Iraq
[2]Dept. of Applied Biotechnology, Al-Qasim Green University, College of Biotechnology, Babylon, Iraq

Abstract

Background: Diabetes mellitus is the most predominant endocrine disorders. Nearly, 1.4 million patients have been diagnosed with diabetes in Iraq. This disease can cause a weakened immune system leading to increasing risk of infections with many microbial diseases.
Aim and Objective: The aim of this study was to determine the yeast structure in the oral cavity of patients with and without diabetes mellitus and the potential association between serum glucose level (HbA1c) and oral Candida carriage in diabetics via phenotypic and molecular identification, and phylogenetic analysis of isolated yeasts.
Materials and Methods: Fungal survey on 64 individuals (age 20-72 yrs.) was divided into two groups 34 diabetic patients and 30 non-diabetic individuals to determine diverse fungal species using molecular and morphological identification isolated from the mucosal oral cavity in both diabetes and non-diabetes in Babylon province, Iraq.
Results: The results showed that there were nine common species including *Candida albicans*, *Candida dubliniensis*, *Candida Africana*, *Kluyveromyces marxianus*, *Kluyveromyces* sp., *Yarrowia* sp., *Wickerhamomyces anomalus*, *Nakaseomyces glabratus* and *Naganishia* sp. mostly belonging to Ascomycota in both the diabetic and non-diabetic groups. Molecular and morphological tools such as the Maximum likelihood phylogenetic analysis and chromogenic media confirmed the identification of these fungi. The isolated fungi were more diverse and frequent in the diabetes patients compared with non-diabetes. *C. albicans* was the most common species followed by the species *Kluyveromyces marxianus*.
Conclusion: These findings provided significant insight into acknowledgment about the distribution and diversity of commensal yeast in the oral cavity in Babylon province, Iraq, and the risks of expending infections due to alterations in compositions of the microbial communities regarding to health issues.

Keywords: Mycobiota, Yeast, Candida, Mucosal oral, Diabetes, Phylogenetic analysis.

Received: 02-01-2025; **Accepted:** 02-03-2025; **Available Online**: 29-03-2025

1. Introduction

Diabetes mellitus (DM) is the most predominant endocrine disorders due to dysfunction of beta cells in pancreatic islet 1 leading to higher levels of glucose plasma for a long period.[1,2] Globally, this fastest-growing disease is considered a vast threat to human health and affects millions of individuals around the world.[3] Diabetes have reached frightening ranks with 537 million adults all around the world living with a rising prevalence worldwide.[4,5] Females are projected to have somewhat lower prevalence of diabetes than men. Men contribute 17.7 million more than female diabetes in 2021.[5] This health issue causes changes in the oral environment leading to alterations in microbial colonization of the oral cavity.

The oral cavity is a major entry point for microbes into the body. That part is histologically lined via mucosal epithelial cells and constantly filled with saliva secreted via different salivary glands. The features of the oral mucosal cavity contribute to activate several mechanisms of many beneficial microbes.[6] The normal mucosal surface of the oral cavity is resident with a varied and multifaceted structure of the microbiota including bacteria and fungi. That is due to their diverse physiological activity and their abilities to adhere to the mucous membranes in some organisms.[7] The oral microbiota mainly contributes to the human health and

*Corresponding author: Rajaa A. A. Al Anbagi
Email: ralanbagi@biotech.uoqasim.edu.iq

https://doi.org/10.18231/j.ijmr.2025.016

influences diseases. There has been limited data considering how the oral microbes' network together and influence the host immune system.

Many studies have been characterizing the oral microbiota focusing on the bacterial community. However, few encouraged studies have been detecting the oral mycobiota. Fungi, yeasts specifically, can commensally colonize the oral cavity without affecting the host's health but also can be opportunistic pathogens when there is a change in the balance of the resident microbiota and in the immune system.[8-10] *Candida* species as oral residents, for example, are able to threaten various organs and cause disease in both immunocompromised and seriously ill patients.[10] The oral mycobiota alters throughout life and is affected by several host lifestyles and related factors including oral hygiene, dietary, and immunological function.[11] Generally, *Candida* species represent up to 60% of the oral flora of healthy individuals without any specific signs or symptoms.[8,12] Several *Candida* species have been isolated from the oral cavity as a commensal, but their prevalence was different in non-diabetic patients due to increasing levels of glucose in saliva leading to the transition to pathogens causing yeast infections. However, *C. albicans* is reported to be the most prevalence in the diabetic individuals comprising 56% compared to in 30% non-diabetic individuals.[12]

In Iraq, it has nearly 1.4 million patients with diabetes.[13] This disease causes a weakened immune system leading to an increasing risk of infection with many microbial diseases as being recorded with the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 and its consequences.[14]

However, little data has been reported about yeast flora and the etiology of yeast infections in both healthy and infected individuals in Babylon, Iraq. Thus, the presented study aimed to assess the yeast structure in the oral cavity of patients with diabetes mellitus compared to individuals without diabetes. Furthermore, the potential association between serum glucose level (HbA1c) with the oral *Candida* carriage and in diabetics were also investigated in comparison with healthy individuals. Molecular identification of the isolated yeasts was also detected.

2. Materials and Methods

2.1. Study samples and determination of cumulative glucose levels HbA1C

A laboratory-based descriptive research was performed during the period of May 2022 to May 2023. The study samples' group included 64 individuals of different ages ranging from 27 to 70 years who arrived at some laboratories in Babylon, Al-Hillah City. This group was assembled into two sets. The first group consisted of 34 diabetic patients with type II diabetes mellitus (18 females and 16 males). Another was the control group included 30 healthy persons (18 females and 12 males).

The blood samples of participants in both groups were collected for the estimating of glycated hemoglobin (HbA1c) using the absorption spectroscopy technique reflecting the cumulative glucose exposure of erythrocytes over the last 90 days. The absorption spectra of glycated hemoglobin (HbA1c (%). Samples were recorded in the spectral range 200-850 nm using an optic fiber-based Single Beam UV VIS Spectrophotometer (Ocean Optics CHEMUSB4-UV-VIS single beam spectrophotometer).[15] The glycated haemoglobin values were obtained after being estimated using the absorption spectrum.

All cases were interviewed, and data was recorded using specially designed questionnaires that included demographic data on name, age, being smoking, medical history of the present diseases, any candida disease symptoms and antibacterial or antifungal therapy for the previous month. The individuals of both groups were not smoking.

2.2. Sample collection and laboratory processing of samples

Sterile swabs were used to obtain samples from the oral cavity of each participant. These were immediately placed in a sterile tube and transported in ice bags to the laboratory. Then, the swabs were cultured on Sabouraud dextrose agar (SDA) medium (Merck, Germany). The cultured plates were incubated at 37 °C for two weeks with a regular examination until fungal colonies appeared or a negative result was confirmed. Later, the pure colonies were re-cultured on CHROMagar *Candida* (HiMedia, Mumbai, India) for the isolation and primary identification of *Candida* species. The medium was prepared according to the manufacturer's instructions. The *Candida* isolates were streaked on CHROMagar *Candida* and identified after incubation for 48 h at 37°C. Later, based on the manufacturer's instructions, the colours of the developed colonies were recorded.[16] These tests have been extensively utilized for quick identification of *Candida* species closely related to *C. albicans* based on their colony colour on this medium.[17,18] Colonies with different morphological features or colours were transferred in SDA slants and storage for molecular identification.

2.3. DNA extraction and PCR amplicon sequencing

The whole genomic DNA of purified isolated species was extracted and purified using genomic FavorPrepTM Fungi/Yeast Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., South Korea) based on the manufacturer's instructions. Then, samples of DNA were preserved in deep freeze at -20°C until used in PCR amplification. Nuclear ITS primers, ITS1 and ITS4 related to the conserved nucleotides from the ITS regions have amplified rDNA from presented species. The PCR prepared mixtures, programs, and visualization of PCR products were performed as being described in Al Anbagi *et al.*[19] The PCR products of amplified DNA were selected for sequencing using the

Sanger sequencing technique (Macrogen Company, South Korea).

2.4. Bioinformatic and statistical analysis

The acquired forward and reverse sequences of ITS were integrated using Geneious software (V 9.1.8) (Biomatters Ltd., Newark, NJ). The consensus sequences were blasted against the global databases using BLASTn queries in NCBI to obtain preliminary identifications with a high similarity to type specimens' sequences. Later, the ITS sequences of the identified twenty-two sequences of the fungal species were deposited in GenBank under the accession numbers PQ222640 to PQ222661. Maximum likelihood analyses were performed in Geneious version 9.1.8 using PhyML Version 7.2.8[20] and quick bootstrapping for 1000 replicates with the GTR model of nucleotide replacement. A phylogenetic tree was visualized in Geneious version 9.1.8.

3. Results

The estimating of glycated hemoglobin (HbA1c) in the blood samples in both groups using the absorption spectroscopy technique ranged 6.5-16% for diabetes participants and 4.1 to 6.4% for non-diabetes participants (**Figure 1**).

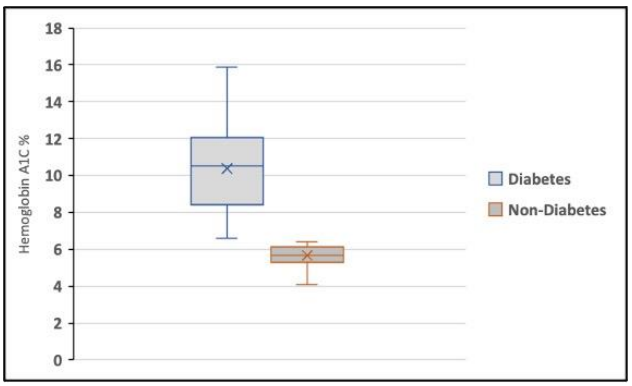

Figure 1: The ranges of percentages of A hemoglobin A1C (HbA1C %) in blood of two studied groups

The present results include data from a total of sixty-four swabs collected from the oral cavities of individuals without any symptoms of fungal infections. The results showed diverse species isolated from the mucosal oral cavity in both diabetes and non-diabetes samples. In total, 97.37% and 89.66% of the samples from the diabetic and non-diabetic groups respectively had Ascomycota mostly *Candida* species while the rest of these percentages belonged to Basidiomycota represented by only one species, *Wickerhamomyces anomalus* (**Figure 2**). The oral examinations showed there were no clinical infections in both investigated groups representing that the colonization was subclinical confirming the previous results that 3-75% of the oral carriage rate normally involves *Candida* species without any sign of disease.

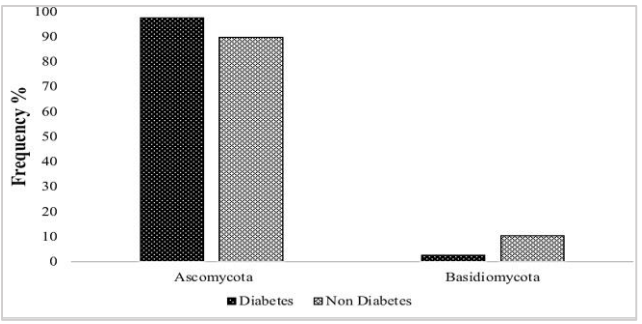

Figure 2: The resident mycobiota based on phyla of the oral mucosal surfaces of both diabetes and non-diabetes.

In the current results, although diverse species were isolated from the mucosal oral cavity in both diabetes and non-diabetes of Iraqi samples, the non-diabetic individuals, controls, were more species richness with nine species compared to diabetic patients who had seven species (Figure 3). These nine species are *Candida albicans*, *Candida dubliniensis* and *Candida Africana*, *Kluyveromyces marxianus*, *Kluyveromyces* sp., *Yarrowia* sp., *Wickerhamomyces anomalus*, *Nakaseomyces glabratus* and *Naganishia* sp. (**Figure 3**).

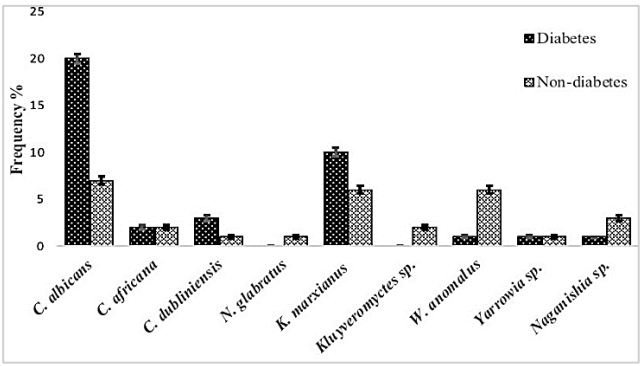

Figure 3: The frequency of resident species of the mycobiota on the oral mucosal surfaces of both diabetes and non-diabetes.

The result of morphological features and chromogenic media, the CHROMagar *Candida* show that yeast species have been confirmed for the identification of *Candida* species (**Figure 4**).

Furthermore, the result of Maximum Likelihood phylogenetic analysis reveals that all sequenced fungal isolates clustered with type materials of known specific fungal specimens (**Figure 5**). All *Candida* species separated in a monphylogenetic clade from all other fungal species and clustered with their type specimens. Furthermore, the fungal isolates C21, C22, C25, C28, C44, and C4a1 clustered as *K. marxianus* with the type specimen of *K. marxianus* while C53 cluster as uncommon *Kluyveromyces* species. The fungal isolates C52, C33, C36 grouped with type specimen of *Yarrowia* sp. *Wickerhamomyces anomalus* and *Nakaseomyces glabratus* respectively. The molecular

identification became a powerful tool to confirm identifying common and cryptic fungal species.

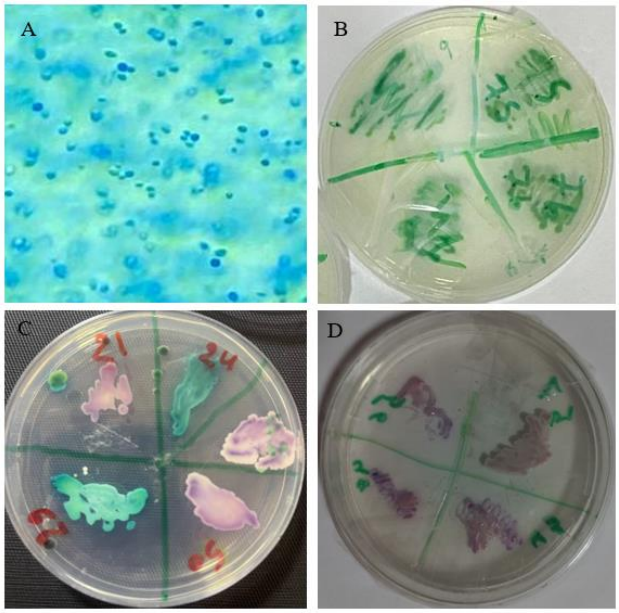

Figure 4: Morphological examinations of some *Candida* species. (A) Microscopic examination. (B) Colonies grown on CHROMagar *Candida* for 48 h at 30°C for *Candida albicans*, (C) *K. marxianus*, and (D) *C. glabrata* (*Nakaseomyces glabratus*).

Additionally, the current results revealed the higher numbers of colony forming unites were recorded in diabetes for *Candida albicans* (474 colony) followed by *Kluyveromyctes marxianus* (311 colony) whereas *C. dubliniensis* (132 colony), *C. Africana* (71 colony) and *Naganishia* sp. (30 colony) were highest in non-diabetics, controls (**Figure 6**). In non-diabetics, the numbers of colonies distributed mostly evenly. The yeast community structure of non-diabetic individuals was more diverse with recording 9 species while recording 7 species only in diabetic people (**Figure 3**).

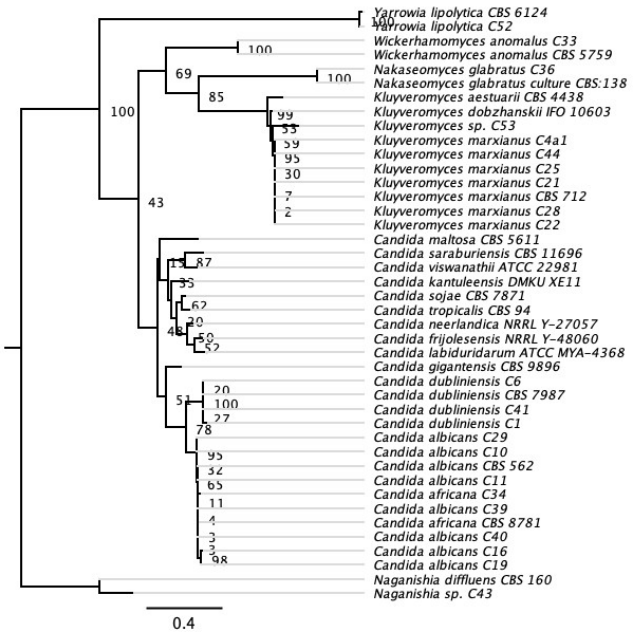

Figure 5: ITS phylogeny of some Iraqi yeast species using the Maximum Likelihood phylogenetic analysis. The nodes display Bootstrap support values. All other sequences use beside the sequences of this study are recovered from type materials.

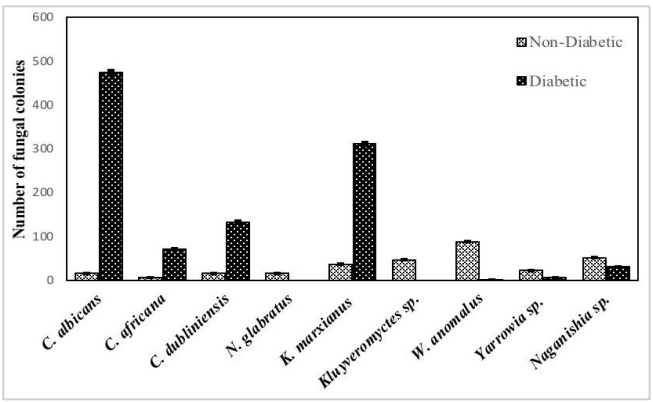

Figure 6: Total colony forming units of fungi within diabetes and non-diabetes.

Table 1: Fungal diversity in the study samples.

Name	Mean	Stand.Dev.	Sum	S	E	H	D`
Candida albicans	7.66	20.95	490.0	27	0.68	2.24	0.87
Candida Africana	1.20	6.24	77.0	4	0.68	0.95	0.57
Candida dubliniensis	2.30	11.31	147.0	4	0.78	1.09	0.61
Nakaseomyces glabratus	0.23	1.88	15.0	1	0.00	0.00	0.00
Kluyveromyces marxianus	5.42	18.33	347.0	16	0.66	1.84	0.80
Kluyveromyces sp.	0.72	4.03	46.0	2	1.00	0.69	0.50
Wickerhamomyces anomalus	1.39	6.11	89.0	7	0.66	1.28	0.69
Yarrowia sp.	0.44	2.84	28.0	2	0.75	0.52	0.34
Naganishia sp.	1.27	5.31	81.0	4	0.94	1.31	0.71
Averages	2.29	8.55	146.7	7.4	0.69	1.10	0.56

S = Richness; E = Evenness; H = Shannon`s diversity index; D = Simpson`s diversity index.

Figure 7: Diagrams of percentiles of colony forming unites based on gender and family taxonomic levels.

Moreover, the current results evaluated the influence of gender on the yeast community structure (**Figure 7**). Based on gender, both male and female had the same species except the female consisted of additional species, *Candida africana*. In diabetic patients, *C. albicans* was the highest frequency at 45% followed by Kluyveromyces *marxianus* 16% in DM males while *K. marxianus* 16% after that *C. albicans* 7% in DM females. In non-diabetic, females were more diverse with seven species. In that community, *Wickerhamomyces anomalus* was the highest frequency at 29% followed by *Naganishia* sp. However, males' yeasts were less diverse involving only five species. The *Naganishia* sp. comprised 15% from that community followed by *Kluyveromyces marxianus* at 13%.

4. Discussion

Fungal colonization of the human body has been studied extensively due to the significance of the fungi as opportunistic pathogens that result in an important health issue for patients especially those with a weakened immune system.[21,22] Studying the association of medical fungi with human body without symptoms could provide warranting of risks of these organisms. Many diseases could cause a weakened immune system. Among these diseases, diabetes mellitus (DM) type II can cause an immunosuppressive effect on patients. In this work, the oral examinations showed there were no clinical infections with any medical fungi in both investigated groups representing that the colonization was subclinical. These results confirmed the previous outcomes that 3-75% of the oral carriage rates normally involve *Candida* species without any signs of a disease. However, fungi were detected in 97.37% and 89.66% of the current samples from the diabetic and non-diabetic groups respectively with the presence of some common fungal species. Other studies partially confirmed the results of this study that high percentages of several *Candida* spp. in diabetic patients such as 75%, 57%, and 5% of *C. dubliniensis*, *C. albicans*, and *C. glabrata* respectively, are higher than in non-diabetic individuals which were 20% and 14% of *C. albicans* and *C. dubliniensis* respectively using PCR experiments.[23-26] Likewise, *Candida albicans* and *Kluyveromyctes marxianus* were the most diverse among detected fungi in our overall samples (**Table 1**). Other studies that compared the fungal communities between diabetic and non-diabetic participants confirmed that *Candida* colonization in the oral mucosal cavity was higher in diabetic patients than non-diabetic individuals.[27-29] Species of *Candida* have been recovered frequently from the diabetic patients who have increasing levels of glucose in saliva.[30] Many factors may affect the colonization *of Candida* in the mouth cavity of diabetes such as the duration of diabetes or the patient's age, modes of treatment, and smoking.[31] Furthermore, compared with diabetic non-pregnant women, diabetic pregnant women have a higher prevalence of *Candida* infections. That is because of the high levels of blood glucose in diabetes lead to suppress the immune system during pregnancy.[32] Additionally, it is presumed that the high level of glucose could serve as a source of the carbohydrate energy of *Candida* sp. that helps to form biofilm and could be required to produce the polysaccharide matrix.[23] This matrix, secreted by sessile cells, could assist to challenging the inappropriate environmental conditions.[33]

In these communities, the highest percentages of fungal frequency were *Candida albicans* in diabetic sets (**Figure 2**). In contrast with another study, *C. albicans* is the most common colonizing species from the human oral cavity in both health and disease.[6] However, the highest frequency of *C. albicans* was in diabetics indicating they have a high risk

of the oral candidacies and the invasive candidiasis.[34] Although *C. albicans* are considered commensal flora, diabetic patients are at a high risk of progressive and severe periodontitis compared with healthy individuals. The reduction in salivary flow rate and increased salivary glucose concentration in diabetics may contribute to increased risk of developing periodontitis.[37] The Iraqi patients have the highest levels of glucose in serum. This increasing triggers distinctive consequences, starting from damaging different types of cells including human defences such as epithelial cells, monocyte and neutrophil adherence and phagocytosis to disturbing the fungal balance and invasion of the infected tissues.[35] The physiological and hormonal disorders of diabetes mellitus led to changing the oral health histologically, immunologically, and microbiologically.[6] For example, reduction in saliva secretion and immunoglobulin action increase the risk of fungal infections due to their protective functions and the first-line oral defense.[36]

The current results concurred with previous results that *C. albicans* are the most common species able to transition from commensal to pathogens in the oral cavity of diabetics due to changing some factors including condensed salivary flow, higher salivary glucose levels, and weakened candidacidal activity of neutrophils.[37] Later, the abnormal conditions effectively increase the exoenzymic activity of *Candida* species such as hemolytic, esterase, and phospholipase leading to digestion and destruction of the oral mucosal surfaces. These process initiate cell lysis and enable fungal penetration.[30] A previous study also confirmed that the *Candida* spp. were higher in diabetic patients and the *Candida albicans* (36.2%) was the most frequent species associated with the oral cavity. However, *C. albicans* was also the most common species (27%) in the non-diabetic patients.[38]

Although *Candida africana* from a patient with vaginitis can be determined physiologically and morphologically from *C. albicans* but there is a confusion to separate them genetically using the analysis of 26S rRNA gene with 100% homology. Therefore, it was believed that *C. africana* represents atypical isolates of *Candida albicans*.[39] The phylogenetic relationship of *C. africana* with other closely related *Candida* species is still controversial.[40] However, the genetic tool used to identify fungi is still the best method to approve the identification.[41]

The influence of gender on the yeast community structure was observed. Although the number of species generally associated with females and males was slightly different, in diabetic patients, the *C. albicans* was the highest percentage in the males followed by *K. marxianus*, while in male patients, *C. albicans* was lower than *K. marxianus*. However, other species were the highest percentage in non-diabetic patients (**Figure 7**). In the different result, *C. albicans* was the most common species (87.5%), while in diabetic females, dermatophytes were more prevalent (58.3%) in diabetic males.[42] Furthermore, diabetic females had the higher percentage of fungal infection (85.7%) than fungal infection in diabetic males (60.7%).[43]

5. Conclusion

It is concluded that the commensal yeasts colonized oral samples with mostly candidal colonization in diabetes and non-diabetes individuals in Babylon samples. Although species richness is high in non-diabetes individuals, high candidal colonization by some *Candida* spp. was detected in diabetes patients such as *C. albicans* and *K. marxianus*. These commensal species could be able to transition from commensals to pathogens in the oral cavity of diabetics. Gender could have effects on the structure of the yeast community.

6. Ethical Approval

This study was approved by the local ethical committee, the Al-Qasim Green University with ref. no. 8102022.

7. Source of Funding

This study was funded by contributed researchers.

8. Conflict of Interest

The authors declare no conflict of interest to the present study.

References

1. Bakhti M, Böttcher A, Lickert H. Modelling the endocrine pancreas in health and disease. *Nat Rev Endocrinol*. 2019;15(3):155–71.
2. Martorano-Fernandes L, Dornelas-Figueira LM, Marcello-Machado RM, Silva RD, Magno MB, Maia LC, et al. Oral candidiasis and denture stomatitis in diabetic patients: Systematic review and meta-analysis. *Braz Oral Res.* 2020;34:e113.
3. Kumari G, Singh V, Chhajer B, Jhingan AK. Effect of lifestyle intervention holistic approach on blood glucose levels, health-related quality of life and medical treatment cost in type 2 diabetes mellitus patients. *Acta Sci Health Sci*. 2021;8(43):e53729.
4. Mauri-Obradors E, Estrugo-Devesa A, Jané-Salas E, Viñas M, López-López J. Oral manifestations of Diabetes Mellitus. A systematic review. *Med Oral Patol Oral Cir Bucal*. 2017;22(5):e586–94.
5. International Diabetes Federation. IDF Diabetes Atlas [Internet]. 10th ed. Brussels: International Diabetes Federation; 2021 Available from: https://www.ncbi.nlm.nih.gov/books/NBK581934/
6. Lenka S, Swain SK, Bhuyan R, Sahu MC. Fungal infection in the oral cavity: A review. *Int J Cur Res Rev,* 2020;12(18):149–53.
7. Chandra J, Retuerto M, Mukherjee PK, Ghannoum M. The Fungal Biome of the Oral Cavity. *Methods Mol Biol*. 2016;1356:107–35.
8. Samaranayake L. Commensal oral *Candida* in Asian cohorts, *Int J Oral Sci*. 2009;1(1):2–5.
9. Janus MM, Willems HM, Krom BP. Candida albicans in multispecies oral communities; a keystone commensal? *Adv Exp Med Biol*. 2016;931:13–20.
10. Magalhães J, Correia MJ, Silva RM, Esteves AC, Alves A, Duarte, AS. Molecular techniques and target selection for the identification of *Candida* spp. in oral samples. *Appl Sci.* 2022;12(18):9204.
11. Samaranayake L, Matsubara VH. Normal Oral Flora and the Oral Ecosystem. *Dent Clin North Am.* 2017;61(2):199–215.
12. Amin AM, Sadiq NS, Saeed CH. Isolation of *Candida albicans* from oral cavity of type II diabetic subjects and its relationship to total

and differential white blood cell count. *Zanco J Med Sci*. 2014;18(3):833–8.
13. World Health Organization. Diabetes. Geneva: World Health Organization; 2018. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes.
14. Ibrahim SL, Alkhakany AJ, Mizil ZF. Investigation of Risk Factors for Hospitalization of COVID-19 Patients with Diabetes in Najaf, Iraq. *Arch Razi Inst*. 2022;77(5):1639–45.
15. Mallya M, Shenoy R, Kodyalamoole G, Biswas M, Karumathil J, Kamath S. Absorption spectroscopy for the estimation of glycated hemoglobin (HbA1c) for the diagnosis and management of diabetes mellitus: a pilot study. *Photomed Laser Surg*. 2013;31(5):219–24.
16. Jabra-Rizk MA, Brenner TM, Romagnoli M, Baqui AA, Merz WG, Falkler WA Jr, et al. Evaluation of a reformulated CHROMagar Candida. *J Clin Microbiol*. 2001 May;39(5):2015–6.
17. Tietz HJ, Hopp M, Schmalreck A, Sterry W, Czaika V. *Candida africana* sp. nov., a new human pathogen or a variant of *Candida albicans*? *Mycoses*. 2001;44(11-12):437–45.
18. Fotedar R, al-Hedaithy SS. Identification of chlamydospore-negative *Candida albicans* using CHROMagar Candida medium. *Mycoses*. 2003;46(3-4):96–103.
19. Al Anbagi RA, Alshuwaili FE, Stephenson SL. Fungi associated with forest floor litter in northwest Arkansas. *Curr Res Environ Appl Mycol*. 2019;9:25–35.
20. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol*. 2010;59(3):307–21.
21. Rodrigues CF, Rodrigues ME, Henriques M. *Candida* sp. infections in patients with diabetes mellitus. *J Clin Med*. 2019;8(1):76.
22. Chyad SA, Al Anbagi RA. Effect of COVID-19 and Its Vaccine: Hematological and Immunological Study of Recovered Individuals Based on Gender. *Med J Babylon*. 2024;21(2):375–82.
23. Javed F, Al-Askar M, Al-Rasheed A, Babay N, Galindo-Moreno P, Al-Hezaimi K. Comparison of self-perceived oral health, periodontal inflammatory conditions and socioeconomic status in individuals with and without prediabetes. *Am J Med Sci*. 2012;344(2):100–4.
24. Javed F, Al-Askar M, Samaranayake LP, Al-Hezaimi K. Periodontal disease in habitual cigarette smokers and nonsmokers with and without prediabetes. *Am J Med Sci*. 2013;345(2):94–8.
25. Javed F, Tenenbaum HC, Nogueira-Filho G, Nooh N, O'Bello Correa F, Warnakulasuriya S, et al. Periodontal Inflammatory Conditions Among Gutka Chewers and Non-chewers With and Without Prediabetes. *J. Periodontol*. 2013b;84:1158–64.
26. Javed F, AlGhamdi TAS, Mikami T, Mehmood A, Ahmed HB, Samaranayake LP, et al. Effect of Glycemic Control on Self-Perceived Oral Health, Periodontal Parameters, and Alveolar Bone Loss Among Patients with Prediabetes. *J. Periodontol*. 2014;85(2):234–41.
27. Kumar BV, Padshetty NS, Bai KY, Rao MS. Prevalence of *Candida* in the oral cavity of diabetic subjects. *J Assoc Physicians India.* 2005;53:599–602.
28. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, et al. Candidal overgrowth in diabetic patients: potential predisposing factors. *Mycoses.* 2005;48(3):192–6.
29. Kadir T, Pisiriciler R, Akyuz S, Yarat A, Emekli N, Ipbuker A. Mycological and cytological examination of oral candidal carriage in diabetic patients and nondiabetic control subjects: thorough analysis of local aetiologic and systemic factors. *J Oral Rehabil.* 2002;29(5):452–7.
30. Nouraei H, Ghaderian Jahromi M, Razeghian Jahromi L, Zomorodian K, Pakshir K. Potential Pathogenicity of *Candida* Species Isolated from Oral Cavity of Patients with Diabetes Mellitus. *Biomed Res Int*. 2021;2021:9982744.
31. Apper-Jones LM, Aldred MJ, Walker DM, Hayes TM. Candidal infections and populations of *Candida albicans* in mouths of diabetics. *J Clin Pathol*. 1981;34(7):706–11.
32. Carrol C, Hurley R, Stanley V. Criteria for diagnosis of *Candida vulvovaginitis* in pregnant women. *J Obstet Gynecol Br Common.* 2003;80(3):258–63.
33. Javed F, Näsström K, Benchimol D, Altamash M, Klinge B, Engström PE. Comparison of Periodontal and Socioeconomic Status Between Subjects with Type 2 Diabetes Mellitus and Non-Diabetic Controls. *J. Periodontol*. 2007;78:2112–9.
34. Russell CM, Schaefer KG, Dixson A, Gray ALH, Pyron RJ, Alves DS, et al. The *Candida albicans* virulence factor candidalysin polymerizes in solution to form membrane pores and damage epithelial cells. *Elife.* 2022;29:11:e75490.
35. Shoham S, Marwaha S. Invasive fungal infections in the ICU. *J Intensive Care Med.* 2010;25(2):78–92.
36. Amerongen AN, Veerman EC. Saliva–the defender of the oral cavity. *Oral Dis*. 2002;8(1):12–22.
37. Kumar S, Padmashree S, Jayalekshmi R. Correlation of salivary glucose, blood glucose and oral candidal carriage in the saliva of type 2 diabetics: A case-control study. *Contemp Clin Dent*. 2014;5(3):312–7.
38. Mohammadi F, Javaheri MR, Nekoeian S, Dehghan P. Identification of *Candida* species in the oral cavity of diabetic patients. *Curr Med Mycol*. 2016;2(2):1–7.
39. Alonso-Vargas R, Elorduy L, Eraso E, Francisco Cano J, Guarro J, Pontón J, et al. Isolation of *Candida africana*, probable atypical strains of *Candida albicans*, from a patient with vaginitis. *Med Mycol*. 2008;46(2):167–70.
40. Romeo O, Criseo G. *Candida africana* and its closest relatives. *Mycoses*. 2011;54(6):475–86.
41. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural products research community. *J Nat Prod.* 2017;80(3):756–70.
42. Chi CC, Wang SH, Chou MC. The causative pathogens of onychomycosis in southern Taiwan. *Mycoses.* 2005;48(6):413–20.
43. Nigotia P, Subha M, Jain S, Jain SK, Kansal A, Garg A. A study of fungal infections in diabetic patients in relation to glycemic status: A prospective study. *J Cardiovasc Dis Res*. 2022;13(5):3328–34.

Cite this article: Al Anbagi RAA, Alshuwaili FRH, Chijo AC, Awad GW. Comparison of mucosal oral fungal colonization between diabetic patients and non-diabetic individuals in Babylon using molecular identification. *Indian J Microbiol Res*. 2025;12(1):106–112.