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Determination of Factors Affecting the Isolation of Candida Species Among Diabetic Patients with Oral Candidiasis in Duhok Governorate, Iraq

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Abstract

Oral candidiasis, a fungal infection primarily caused by Candida albicans, poses a significant health risk for diabetics due to their weakened immune systems and altered oral environments. The prevalence and risk factors for oral candidiasis were evaluated in this cross-sectional study of 367 diabetic patients at Azadi Teaching Hospital in Duhok City from August 2024 to April 2025. Sterile swabs were used to collect clinical samples, which were then cultured on various selective media to identify the species of Candida. According to the results, 34.1% of patients tested positive for Candida species, with the most common species being Candida albicans (13.9%), followed by Candida tropicalis (9.8%) and Candida glabrata (3.0%). A noteworthy correlation was observed between comorbid conditions such as dental problems, high blood pressure, and high cholesterol with oral candidiasis. Additionally, type 2 diabetes and advanced age were prevalent among those affected. The study emphasises the importance of preventive measures and regular oral health examinations to reduce the risk of fungal infections in diabetic patients.

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1. Introduction

Candida is a genus of opportunistic fungi that are frequently present in the oral cavity, vaginal mucosa, and gastrointestinal tract as a natural component of the human microbiota. Certain species, such as Candida albicans, are generally harmless in healthy individuals, but they can cause infections when the host's immune system is weakened or when the microbiome is disrupted [1]. These infections, referred to as candidiasis, can range from minor mucosal infections to potentially fatal systemic candidemia, especially in immunocompromised individuals [2].

Oral candidiasis, often referred to as oral thrush, is a fungal infection caused by Candida species, primarily Candida albicans. This condition arises when immunosuppression, antibiotic use, diabetes, or poor oral hygiene disrupt the normal balance of the oral microbiota [3]. Oral candidiasis manifests as white, curd-like plaques on the tongue, inner cheeks, and other parts of the oral cavity, which can be uncomfortable or unpleasant [4].

The Candida genus comprises various species, some of which can develop into opportunistic infections, while others are commensal microbes in the human microbiome. The most frequent cause of candidiasis in humans is Candida albicans, which can lead to both superficial infections, such as vaginal candidiasis and oral thrush, and systemic infections, including candidemia. Candida glabrata is commonly associated with bloodstream infections (candidemia) [5]. Patients with impaired immune systems, especially those suffering from neutropenia, are frequently infected with Candida tropicalis. Candida parapsilosis is often linked to catheter-associated bloodstream infections, while Candida krusei is commonly associated with infections in immunocompromised individuals, such as cancer patients undergoing chemotherapy. A new multidrug-resistant strain, Candida auris, is responsible for hospital outbreaks worldwide [6]. Numerous studies suggest that individuals with diabetes are at an increased risk of candidiasis. Factors such as decreased immunity, angiopathy, neuropathy, and increased medicinal interventions contribute to the high prevalence of infections among diabetic patients [7]. High glucose levels in diabetic individuals may promote the growth of yeast in the oral cavity, further increasing the risk of fungal infections [8].

The diagnosis of oral candidiasis may be based on the clinical recognition of a specific Candida species. Clinical forms of oral candidiasis include pseudomembranous candidiasis, hyperplastic candidiasis (candidal leukoplakia), atrophic candidiasis (both acute and chronic erythematous forms), and angular cheilitis [9]. In some cases, diagnosing candidiasis can be challenging, as individuals exhibit varying signs and symptoms depending on their age, gender, host immunity, and environmental exposures. However, diagnostic tools such as questionnaires, complete digestive stool analysis (CDSA), and laboratory methods can be used to screen for and confirm yeast infections [10].

Oral candidiasis can now be treated with a variety of topical and systemic medications. Systemic antifungal medications, such as triazoles (fluconazole, itraconazole), are suitable for individuals at high risk of acquiring systemic infections or those who do not respond to or are intolerant to topical therapies. Topical antifungal medications, including clotrimazole, miconazole, amphotericin B, and nystatin, are typically recommended as the first line of treatment for mild cases of oral candidiasis [11]. The purpose of this study is to isolate and identify Candida species from oral candidiasis in diabetic patients from Duhok Province using HI Chrome Agar and to investigate some associated features.

2. Materials and Methods

2.1 Specimen collection

A total of 367 oral samples must be written in the past tense collected from diabetic patients in the morning period at the Azadi Teaching Hospital in Duhok City, between August 2024 to April 2025. After obtaining permission from the patients to participate in this study. The control sample was taken from healthy individuals without oral candidiasis to compare the presence or absence of *Candida* species, colony, morphology, virulence factors, or immuneresponse.

Samples will be collected from patients using sterile cotton swabs (Sterile Cotton Swabs) containing physiological salt solution NaCl for the clinical evaluation of individuals who were diagnosed, after which they will be reviewed by trained medical professionals. Then, to monitor fungal species, they were cultivated on three basic agars: Sabouraud dextrose agar, Sabouraud Dextrose Broth, Nutrient agar, Sabouraud Chloramphenicol agar, HiCrome Candida Differential Agar, and Corn-Meal Agar (CMA). The tongue, buccal mucosa, and labial sulcus were all touched with a sterile transport swab. Patients who visited hospitals in Iraq's Kurdistan region's Duhok province.

2.2 Number of Samples

A total of 367 swab samples were included in this study.

2.3 Sample collection

Swabs were cleaned with saline and then rubbed over the lesion to collect samples from the base of the ulcer. They can be extracted straight from the purulent exudate as well. After being collected for 24 hours, the samples were brought to the lab and cultivated on potato dextrose agar and Sabouraud dextrose agar. The plates were kept at 37°C for 48 hours

2.4 Preparation of Media

The preparation of all used media was done according to the manufacturer's instructions.

2.4.1 Sabouraud dextrose agar

One litre of distilled water should be used to suspend 65 g of the medium.
To fully dissolve the medium, heat for one minute while stirring often.
Autoclave for 15 minutes at 121° C.
Pour into petri dishes or tubes for slants after cooling to 45 to 50°C.

Figure 1. This is a result

2.4.2 Sabouraud Dextrose Broth
30 g of dehydrated medium (BK026) should be dissolved in 1 litre of demineralised or distilled water.
Slowly stir until the mixture dissolves completely. •
Pour into 100 mL vials or 10 mL tubes. •
Autoclave for 15 minutes at 121 °C to sterilise.
The medium should be cooled to room temperature.

2.4.3 Sabouraud Chloramphenicol agar
42.5 g of powder should be dissolved in 1 L of distilled water.
It should then be heated, swirling constantly, until it dissolves completely.
It should then be autoclaved for 20 minutes at 121 ± 3°C.
Gentamicin or chloramphenicol can be added as needed.
Finally, it should be mixed and poured into sterile Petri plates.

2.4.4 HiCrome™ Candida Differential Agar
1. In 1000 millilitres of distilled water, suspend 42.72 grams.
2. Bring to a boil in order to fully dissolve the medium.
3. AVOID AUTOCLAVING.
4. Lower the temperature to 45–50°C.
5. Pour onto sterilized Petri dishes after thoroughly mixin .

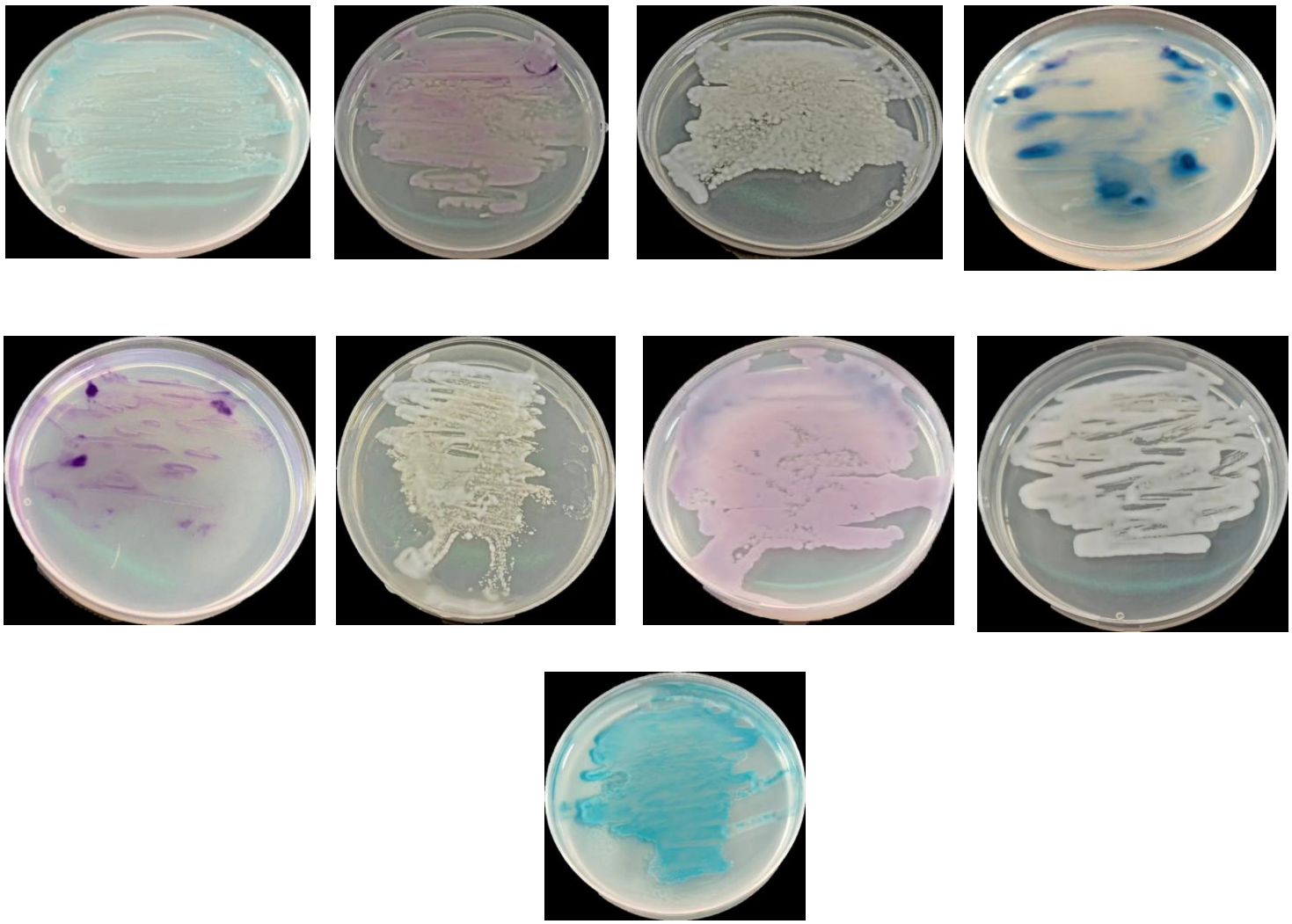


Figure 2. This is a result

2.4.5 Corn Meal Agar (CMA)

This test is a quick way to distinguish between Candida dubliniensis and Candida albicans because it produces fragile, small, tube-like structures called germ tubes after two hours of incubation in human blood serum at 37 oC. In contrast to pseudo-hyphae, germ tubes are elongations of daughter cells from the mother cell without origin shrinking [12].

Virulence factor testing should be performed, ex, proteinase, esterase, hemolysin

The three main enzymes produced by C.albicans are secreted aspartyl protease, phospholipase, and hemolysin [13].

Figure 3. This is a result

2.5 Gram stain

The Gram stain is a differential stain that involves a process called decolourisation, which is the most crucial stage. The data indicate that the cell wall components of Gram-positive (thick peptidoglycan) and Gram-negative (thin peptidoglycan) bacteria determine their ability to withstand decolourisation. Because alcohol or acetone extracts the lipid, the Gram-negative cell wall becomes porous and loses its ability to retain the crystal violet–iodine complex. As a result, the safranin dye can stain it, giving the Gram-negative bacteria a reddish-pink appearance. In contrast, the thick peptidoglycan in Gram-positive bacteria efficiently traps the crystal violet–iodine complex, giving the Gram-positive bacteria a purple appearance and increasing their resistance to decolourisation [14].

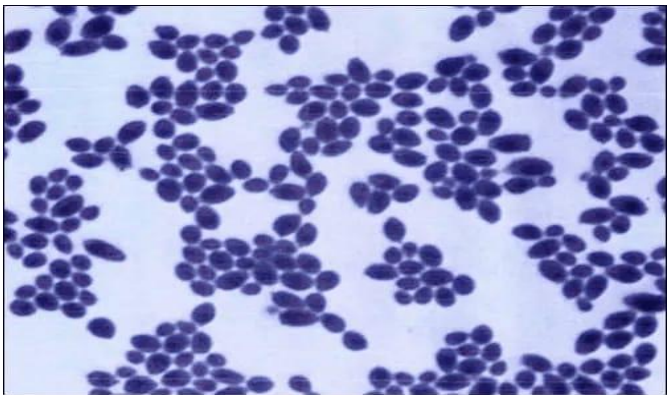


Figure 4. Structure of *Candida* in Gram staining

2.5.1 Germ tube formation test

Candida albicans and Candida dubliniensis may be quickly identified with this test because, when cultured in human blood serum at 37 ´ C for two to three hours, they develop short, slender, tube-like structures termed germ tubes. Because they are elongated daughter cells from the mother cell without constriction at the origin, they are different from pseudo hyphae [15].

Figure 5. germ tube of *Candida albicans*

2.5.2 Antifungal susceptibility test

The antifungal sensitivity test includes several in vitro techniques, such as the minimum inhibitory concentration (MIC), which can be used to assess the activity against certain fungal isolates. The MIC, defined as the lowest concentration of an antifungal agent that will prevent visible growth of a fungus after incubation, is the most widely used laboratory test to determine an antimicrobial's efficacy against fungal species [16]. Antifungal agents that are used in the current study are: Itraconazole, Clotrimazole, Amphotericin B, and Fluconazole.

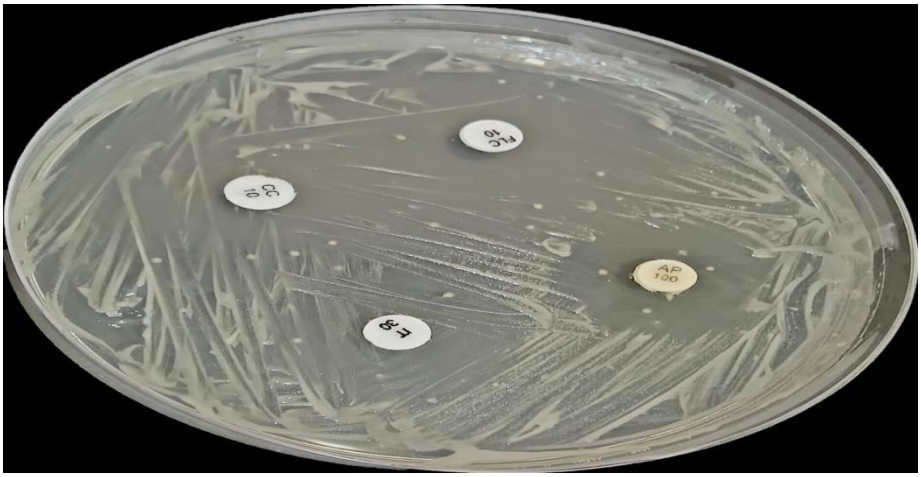


Figure 6. Resulty

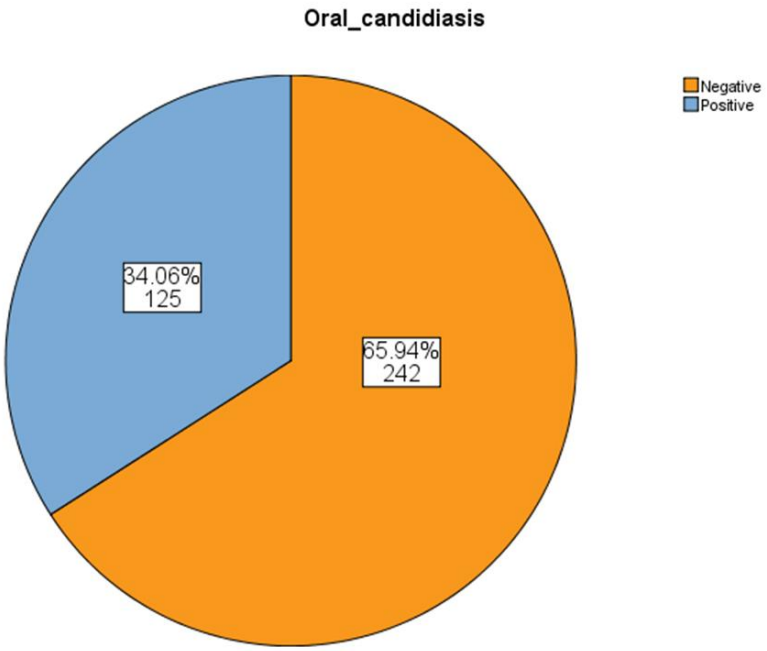
3. The Results

Among 367 diabetic patients (n=125; 34.1%) were positive for *Candida* species (oral candidiasis), and (n=242; 65.9%) were negative, as shown in Graph 1. Of all 367 patients (n=255; 69.5%) were female, and (n=112; 30.5%) were male. Among 367 diabetic patients (n=350; 95.4%) were type 2 diabetic patients, and (16; 4.4%) were type 1 diabetic patients. Most frequent diabetic patients were in the age group 52 – 72 (n=216; 58.9%), and the least wan was the group <=9 years, as shown in Graph 2. The most common *Candida* species isolated from infected patients was *Candida albicans* (13.9%) followed by *Candida tropicalis* (9.8%) and *Candida glabrata* (3.0%), in addition to many other species *Candida utilis* (1.1%), *Candida krusei* (2.7%), in addition to others shown in Table 1. On sabaroud dextrose agar, shown growth of *Candida* species is shown without selecting the type of *Candida,* as shown in Figure 1. Neither on Hichrom Candida agar, most *Candida* species can the identified as the type of *Candida*. Most frequent diabetic patients have High blood pressure, which 339 of 367, and among patients with High blood pressure, 111 were positive for oral candidiasis Table 3. Also, 357 of diabetic patients have high cholesterol levels, 117 of them have positive oral candidiasis Table 4. Also, from diabetic patients, 212 have teeth problems, and 93 of them were positive for oral candidiasis Table 5. Some patients have shortness of breath, and some of them have heart disease, eye problems, surgery, obesity, kidney disease, thyroid disease, and skin problems.

4. Statistical Analysis

SPSS (IBM, version 25) was used to analyse the data. The mean, standard deviation, median, and range were used to express quantitative data. Numbers and percentages were used to present qualitative data.

Graph 1: Presence of oral candidiasis in diabetic patients



Graph 2 : group of age of diabetic patients

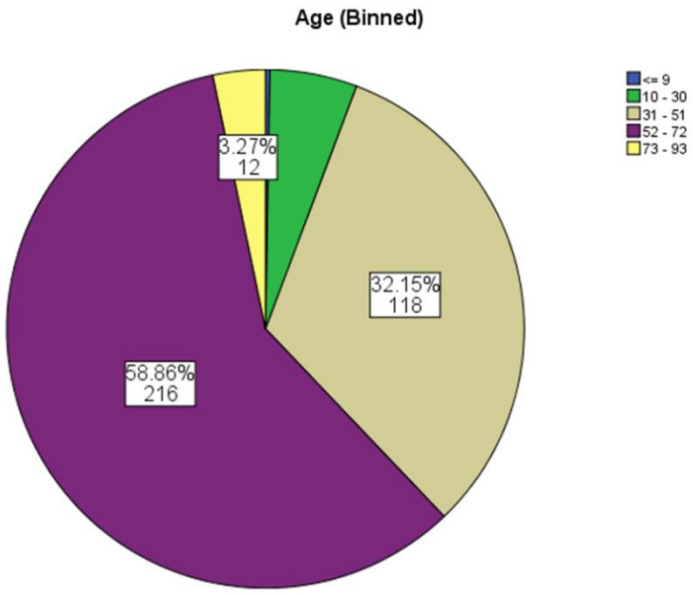


Table 1: Frequency percentage of isolated *Candida* species.

		Frequency	Percent
Valid	Negative	245	66.8
	C. albicans	51	13.9
	C. tropicaliss	36	9.8
	C. glabrata	11	3.0
	C. utilis	4	1.1
	C. krusei	10	2.7
	C. kefyr	3	.8
	C. mixed	1	.3
	C. dubliensis	3	.8
	C. glabrataCh	1	.3
	C. parapsilosis	1	.3
	C. membranifaciens	1	.3
	Total	367	100.0

Table 2: Relationship between variables

		Gender	Type_of_ Diabetes	Years_ Diagnosed	Enus_species	Age (Binned)
Oral_candidiasis	***Correlation Coefficient***	0.078	-0.019	0.017	0.954**	0.057
	Sig.	0.134	0.719	0.750	0.000	0.278

Table 3: High blood pressure

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
High blood pressure	228	111	339
Total	228	111	339

Table 4: High cholesterol level

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
High cholesterol	240	117	357
Total	240	117	357

-Although high blood pressure and hyperlipidemia are not considered direct risk factors for oral candidiasis, their frequent presence in diabetic patients may indicate poor glycemic control. This poor metabolic regulation can compromise immune function and create a favorable environment for fungal overgrowth. Therefore, the association between these comorbidities and oral candidiasis is likely circumstantial rather than causal. This interpretation is supported by several studies that have highlighted the indirect influence of systemic conditions, particularly uncontrolled diabetes, on the risk of oral Candida infections.

Table 5: teeth problems

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Teeth problems	119	93	212
Total	119	93	212

Table 6: Shortness of breath

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Shortness of breath	245	119	364
Total	245	119	364

Table 7: Heart disease

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Heart disease	36	25	61
Total	36	25	61

Table 8: Eye problems

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Eye problems	11	23	34
Total	11	23	34

Table 9: surgery in last 5 years

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
surgery in last 5 years	2	3	5
Total	2	3	5

Table 10: Obesity

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Obesity	2	5	7
Total	2	5	7

Table 11: Kidney disease

Oral_candidiasis Crosstabulation

Count		
	Oral_candidiasis	
	Negative	Total
Kidney disease	1	1
Total	1	1

Table 12: Thyroid disease

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Thyroid disease	7	7	14
Total	7	7	14

Table 13: Skin problems

Oral_candidiasis Crosstabulation

Count			
	Oral_candidiasis		
	Negative	Positive	Total
Skin problems	1	4	5
Total	1	4	5

5. The Discussion

The results of this research show a notable occurrence of oral candidiasis in patients with diabetes, with 34. 1% testing positive for Candida species. This finding corresponds with earlier investigations that indicate people with diabetes have a higher likelihood of experiencing fungal infections, including oral candidiasis [17]. Multiple factors contribute to this situation, such as weakened immune responses, poor control of blood sugar levels, and conditions in the mouth that support the growth and colonisation of fungi [8].

Among the Candida species identified, Candida albicans was the most commonly found at 13. 9%, followed by Candida tropicalis at 9. 8% and Candida glabrata at 3. 0%. This pattern agrees with earlier research that has identified C. albicans as the leading cause of oral candidiasis, thanks to its ability to adhere strongly and
create biofilms [18] [19] The presence of non-albicans Candida species like C. glabrata and C. krusei raises clinical concerns because of their greater resistance to standard antifungal treatments, especially azoles.

The information also reveals that issues like high blood pressure and high cholesterol were common within the group studied and linked to a greater occurrence of oral candidiasis. Among 339 diabetic subjects with high blood pressure, 111 were found to have candidiasis, whereas 117 out of 357 patients with high cholesterol were also positive for it. These results indicate that systemic health issues typically associated with diabetes may weaken the body's defences and promote fungal overgrowth.

Oral hygiene seems to be another important factor; of the 212 patients who reported dental problems, 93 (43. 9%) had oral candidiasis. Poor dental care and oral health issues can disturb the balance of microorganisms in the mouth and harm the mucous membranes, leading to increased vulnerability to fungal growth [20] Moreover, the link between older age, especially in the 52 to 72 age bracket, and higher rates of candidiasis might represent both age-related immune decline and the extended duration of diabetes, which together raise the risk of infections [2] .

The high occurrence of type 2 diabetes (95. 4%) among the participants aligns with global trends, where type 2 diabetes accounts for most cases. The elevated rates of infections in these individuals further emphasise the importance of regular oral health screenings and strategies for preventing candidiasis, especially. For those with additional risk factors such as high blood pressure, abnormal lipid levels, or dental problems.

6. Conclusion

Patients with diabetes have a significantly higher prevalence of oral candidiasis, with Candida albicans being the most prevalent causal species, according to this study. The results emphasise the role that diabetes-related factors, such as poor glycemic control, comorbid conditions (e.g., high blood pressure and high cholesterol), and dental health issues, play in increasing susceptibility to fungal infections. The correlation between higher infection rates and advanced age further highlights the risk for elderly diabetics. These findings underscore the need for heightened clinical awareness of fungal infections in this group, as well as the importance of proactive oral hygiene management and regular oral examinations as part of diabetes care. With early detection and coordinated healthcare measures, patients with diabetes can improve their quality of life and significantly reduce the burden of oral candidiasis.

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8. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication.

9. Funding

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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تحديد العوامل المؤثرة على عزل أنواع الكانديدا بين مرضى السكري الذين يعانون من داء المبيضات الفموي في محافظة دهوك، العراق

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[2] قسم الأحياء، كلية العلوم، جامعة دهوك، العراق.

المستخلص

داء المبيضات الفموي هو عدوى فطرية غالبًا ما تسببها فطريات المبيضات البيضاء، ويشكل خطرًا صحيًا كبيرًا على مرضى السكري نظرًا لضعف أجهزتهم المناعية وتغير بيئة الفم. تم تقييم انتشار داء المبيضات الفموي وعوامل الخطر المرتبطة به في هذه الدراسة المقطعية التي شملت 367 مريضًا بالسكري في مستشفى آزادي التعليمي بمدينة دهوك، في الفترة من أغسطس 2024 إلى أبريل 2025. استُخدمت مسحات معقمة لجمع العينات السريرية، ثم زُرعت على أوساط انتقائية متنوعة لتحديد أنواع المبيضات. ووفقًا للنتائج، أظهرت الاختبارات أن 34.1% من المرضى كانوا إيجابيين لأنواع المبيضات، وكانت الأنواع الأكثر شيوعًا هي المبيضات البيضاء (13.9%)، تليها المبيضات الاستوائية (9.8%) والمبيضات الجلابراتا (3.0%). كما لوحظ وجود علاقة ذات دلالة إحصائية بين الأمراض المصاحبة مثل مشاكل الأسنان وارتفاع ضغط الدم وارتفاع الكوليسترول وبين داء المبيضات الفموي لدى المرضى. كان داء السكري من النوع الثاني والتقدم في العمر شائعين أيضًا. تؤكد الدراسة على أهمية اتخاذ التدابير الوقائية وإجراء فحوصات دورية لصحة الفم للحد من خطر الإصابة بالعدوى الفطرية لدى مرضى السكري.