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EVALUATION OF CANDIDA ALBICANS COLONIZATION IN TYPE 2 DIABETIC PATIENTS IN BAGHDAD, IRAQ, USING EXFOLIATIVE CYTOLOGY, GRAM AND LACTOPHENOL COTTON BLUE STAINING

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Background: Candida is a common commensal fungus found in the oral cavity of healthy individuals. However, various systemic and local factors associated with diabetes mellitus can increase the risk of oral candidiasis. The relationship between diabetes and enhanced Candida colonization has clinical significance, particularly for preventing secondary infections.
Objectives: To determine the prevalence and severity of oral Candida colonization in individuals with Type II diabetes compared to healthy individuals, using exfoliative cytology and laboratory staining techniques.
Results:A total of 260 subjects aged 40–60 years were included, comprising 200 Type II diabetic patients and 60 healthy controls. A significant difference in Candida colonization was observed between diabetic and non-diabetic individuals ($p = 0.009$). In the diabetic group, only 3.5% of males and 4.5% of females showed no colonization. Candida colonization was present in 41.5% of diabetic males and 50.5% of diabetic females, indicating a significantly higher prevalence and severity compared to the control group. No significant sex-based differences were noted in the healthy subjects.
Conclusion: Individuals with Type II diabetes exhibit a higher level of Candida albicans colonization in the oral cavity compared to non-diabetic individuals. Early identification and management of oral Candida in diabetic patients may help prevent its progression to bloodstream invasion and subsequent involvement of the gastrointestinal mucosal lining.

Keywords*: Exfoliative cytology, Diabetic patients, Candida albicans, Colonization, Candidiasis*

INTRODUCTION

Globally, the prevalence of diabetes rose from 3.7% to 10.3% between 1990 and 2022.[1] Although diabetes is usually associated with middle-aged or older adults, type 2 diabetes is much more common in younger individuals[2]. The oral cavity contains a variety of common microbiota, such as the harmless commensal fungus Candida, which is present in 20–40% of healthy people. Reputable research on exfoliative cytology has found that Candida has a prevalence rate of 44-55%, making it a normal flora [3]. Candidasis is an infection brought on by various species of the fungus Candida [4]. Clinical diagnosis of oral candidiasis is based on granular, erosive, and pseudomembranous forms of the infection; the latter is the most prevalent and is characterized by easily removable curd-like plaques [5].

However, significant colonization may occur even in the absence of clinical symptoms [6]. Candida albicans is the species most frequently linked to oral candidiasis. Numerous systemic and local factors can increase the risk of oral candidiasis in people with diabetes mellitus (DM) [7]. High blood glucose levels caused by problems with either the effectiveness or production of insulin, or both, are a hallmark of diabetes mellitus (DM), a chronic metabolic disease. However, because these categories often share characteristics, it can be difficult to tell them apart[8]. It is difficult to apply standard invasive procedures to individuals with diabetes because the disease is unpredictable and blood glucose levels vary greatly. Due to the high frequency of Candida infections, Candida albicans is the species most frequently linked to oral candidiasis.

Recent developments in quantitative techniques have prompted a reexamination of the possible contributions of cytology to oral diagnostic procedures. Considering the disadvantages of invasive techniques, this is particularly crucial. By looking at significant changes in the mucosa of patients with diabetes, clinicians can obtain a more accurate picture of the disease. In addition to improving the representation of diabetes, identifying these changes aids in early diagnosis and screening for diabetics[10].

Diabetes mellitus increases a person's vulnerability to opportunistic infections. These include vaginal candidiasis, dental caries, periodontal and gingival diseases, poor metabolic regulation, and malfunctioning salivary glands [11]. Biofilm builds up in immunocompromised individuals with diabetes and in those with reduced salivary flow, increasing susceptibility to opportunistic infections[12]. This phenomenon may be caused by weakened immune responses and nutritional deficiencies, decreased salivary function and pH, and elevated salivary glucose concentrations that promote commensal organism growth in the oral cavity [13].

People with diabetes mellitus had significantly increased oral cavity levels of Candida species. In the oral mycobiome, Candida albicans is recognized as the predominant fungus species [14]. Other species, such as C. glabrata, C. tropicalis, C. krusei, C. dubliniensis, and C. parapsilosis, have also been reported to exist [15].

This disparity may be caused by the type and duration of the disease, the level of glycemic control, the presence of dental variables, and differences in sample methods [16]. Candida colonization can be confirmed by culture techniques, which take a lot of time. However, oral exfoliative cytology provides a quick and easy chairside method. Exfoliative cytology has recently been found to be a helpful tool for identifying these organisms, despite the fact that culture is the most widely used method [17]. The current case control study aimed to compare the incidence and severity of oral candida colonization in type II diabetes to normal human conditions.

MATERIALS AND METHODS

Participants in a case-control research were chosen at Medical City Hospital in Baghdad, Iraq, using a simple random sampling technique. 200 participants with a diagnosis of type II diabetes and 60 controls without the disease were included in the study; they were matched for gender and age. All subjects gave their informed agreement, and the study received ethical approval.

Each patient provided a thorough medical history that included information on blood sugar levels, age, gender, and the length of time they had diabetes. The participants ranged in age from 40 to 60. Every individual underwent a thorough intraoral examination by a professional dentist. People with clinical signs of oral candidiasis, denture wearers, edentulous patients, people with bad oral habits, people who recently received antibiotic treatment, and people with acute or chronic illnesses, endocrine disorders, immunodeficiency conditions, or nutritional deficiencies were all excluded from the study. 260 patients in all had exfoliative oral cytology, as well data obtained via grams and Lactophenol Cotton Blue staining procedures.

For Exfoliative cytology, we used a moist wooden ice cream spatula, two mucosal scrapings were taken from the surface of the tongue and the buccal mucosa. To create smears, these samples were then spread out in a circular pattern on glass microscope slides. In a coupling jar filled with 95% ethyl alcohol, the resultant smears were promptly fixed. Giemsa and Gram stain were used to stain the smears after fixing.

Quality control

Staining reagents and methods have been standardized through the use of peripheral blood smears as a positive control in quality control. A 40× magnification binocular research microscope was used to check each dyed slide for Candida. On a pale green backdrop, the Candida structures appear as magenta to scarlet formations.

The scoring criteria for Candida colonization were established based on its morphological characteristics, as presented in ***Table 1***.

Table 1. Assessment criteria for the presence of Candida colonization

Score	Inference
	There is no indication of Candida
1	The existence of yeast and hyphae, but without any budding
2	A large number of yeast and hyphae with present of budding
3	Numerous yeast and hyphae, characterized by pseudo hyphae ≤3in number
4	A large population of yeast with pseudo hyphae ≥3 in number and branching

These criteria encompass a spectrum from the absence of Candida colonization to the abundant presence of yeast and pseudo-hyphae. Additionally, *Table 2*. outlines the scoring values and grades associated with the levels of Candida colonization.

Table 2. Grades of *candida* colonization

Value	Grades
	No colonization
1	Mild
2 &3	Moderate
4	Severe

RESULTS

Numerous Candida morphological forms, such as genuine hyphae, budding yeast, pseudo-hyphae, branching hyphae with budding yeast, and germ tubes, were seen under light microscopy.

Table 3. Comparison of *candida* colonization between diabetic patients and normal individuals by exfoliative cytology

Normal individuals N (%)	Diabetics N (%)	Candida colonization
42 (70)	66 (33)	Mild
12 (20)	44 (22)	Moderate
6 (10)	90 (45)	Severe
60 (100)	200 (100)	Total

Compared to just 10% of non-diabetic persons, 45% of diabetes patients had a considerable prevalence of severe

Candida colonization. Moderate colonization was found in 22% of diabetic participants and 20% of healthy subjects in the groups under study. On the other hand, Table 3 shows that only 33% of diabetes individuals had mild colonization, compared to 70% of the normal population.

Tables-4 and 5 depicted the colonization rates obtained by Grams stain as well as Lactophenol cotton blue Comparable figures were noticed by the three methodologies practiced in this work.

With a p-value of 0.009, a statistical study using the chi-square test revealed a significant difference in the prevalence of Candida colonization between diabetes patients and healthy people.

Table 4. Comparison of *candida* colonization between diabetic patients and normal individuals by gram staining.

Normal individuals N (%)	Diabetics N (%)	Candida colonization
44(73.3)	69 (34.5)	Mild
15 (25)	53(26.5)	Moderate
1(1.6)	78 (39)	Severe
60 (100)	200 (100)	Total

Table 5. Comparison of *candida* colonization between diabetic patients and normal individuals by Lactophenol Cotton Blue staining

Normal individuals N (%)	Diabetics N (%)	Candida colonization
43(71.6)	70 (35)	Mild
15 (25)	50 (26.5)	Moderate
2(3.3)	80 (40)	Severe
60 (100)	200 (100)	Total

An examination of the prevalence of Candida colonization in relation to gender differences was done and the results are shown in table 6. Gender disparities had no effect on colonization in the non-diabetic (control) group. Only a small percentage of the diabetic group exhibits no colonization (3.5% and 4.5%, respectively). Regarding the diabetic group, candida colonization was seen in 50.5% of females and 41.5% of men. The differences were substantial.

Table 6. Gender Differences in Oral Candida Colonization among Type 2 Diabetic and Non-Diabetic Individuals

Subjects	No. of Cases with Oral *Candida* Carriage	No. of Cases without Oral-*Candida* Carriage	Total
Non-diabetic			
Female	5 (8.8)	26 (91.2)	31
Male	4 (6.6)	25 (93.4)	29
Total	9 (15.4)	51 (83.3)	60
Type 2 Diabetic			
Female	101 (50.5)	9 (4.5)	110
Male	83(41.5)	7 (3.5)	90
Total	184 (92)	16 (8)	200

DISCUSSION

Fungi are common eukaryotic microbes because of their ability to decompose organic materials. Of the more than 100,000 fungal species that science is aware of, only 150 are thought to be intrinsically dangerous. Candida albicans, a commensal yeast that is typically non-pathogenic, is the species most commonly isolated from the oral cavity. These findings align with a study by Mrudula [18], who found that the incidence of candida colonization was significantly higher in diabetic subjects than in healthy individuals.

The validity of our findings is supported by the findings of Alberto Rodríguez et al. [19], who found that people with uncontrolled diabetes had higher levels of Candida colonization. Furthermore, our study shows that oral epithelial cells with probable type 2 diabetes exhibit noticeable morphological changes that increase their vulnerability to candida infections. These changes were successfully identified by microscopic inspection and analysis using the exfoliative cytology of both oral mucosa and tongue methods.

The oral yeast invasion was thoroughly examined in this cytological analysis. The prevalence of candidiasis was particularly high in the group with diabetes compared to the group without the condition. These results are identical with the authors' previous research [20,21]. Other studies, however, discovered varying rates of Candida transmission into the oral cavity. This could be as a result of the use of various collection methods, such as swabs, water, and buffer solutions. Using a wooden spatula, oral and tongue scraping was used in this study to sample the tongue surface and buccal mucosa due to its high sensitivity in determining the transmission of oral candidiasis [22].

Furthermore, because Candida albicans is not evenly distributed in the oral cavity of both healthy and diabetic patients, as this study showed, multiple site oral extraction is useful in assessing the quantitative and total transit of yeast. On the other hand, in some regions, the exchange is better suited to assess the prevalence of candidiasis[23]. Salivary glucose levels and Candida colonization rates are strongly positively correlated, according to the investigation's findings. This result is in line with other research that discovered a link between higher salivary glucose levels and a higher Candida prevalence [24]. The results of our investigation demonstrate that normal salivary glucose levels have no appreciable effect on dental health or bacterial growth. However, it has been demonstrated that higher salivary glucose levels promote Candida's adherence to buccal epithelial cells.

During hyperglycemic episodes, salivary glucose undergoes a chemical reaction with the proteins in tissues to form reversible glycosylated molecules, which accumulate in the buccal epithelium. More Candida receptors may become accessible as a result of this accumulation, which would facilitate Candida's colonization of the tooth pulp and oral mucosa. Moreover, glucose promotes colonization by feeding on Candida species and reducing neutrophils' ability to eradicate these microorganisms. The potential consequences of elevated salivary glucose in diabetics should be considered [25].

The study's findings demonstrated that the oral cavities of diabetics, both male and female, contained more Candida than those of people without the condition. Nevertheless, some studies have not discovered any significant correlation between the incidence of diarrhea and the prevalence of diabetes [26]. This implies that a significant rise in the prevalence of candida may not be primarily due to diabetes mellitus, but rather to specific geographic conditions.

Diabetics are more likely to have Candida in their dental cavities than healthy people. However, in diabetics with dentate diabetes, the amount of yeast on the mucosa remains within normal limits because it is regulated by masticatory movements, salivary secretion, and epithelial turnover. All groups contained both dentate and edentulous patients, which might have increased the incidence of candida colonization. Conversely, those with a history of smoking and those with oral mucosal diseases were not included in this study demonstrates how uncommon candida is in healthy individuals. On the other hand, diabetic individuals had noticeably greater candidiasis rates than the control group [29]. Additionally, the current study showed that compared to individuals with moderate or well-managed diabetes, those with poorly controlled diabetes had greater incidence and levels of Candida.

According to the results, 91.2% and 93.4% of the participants who were not diabetics did not have candida, which Additionally, the current study showed that compared to individuals with moderate or well controlled diabetes, those with poorly managed diabetes had greater incidence and levels of Candida. These results are good in agreement with other studies that found a correlation between the degree of yeast colonization of the oral mucosa, which is typically inhabited by yeast, and the degree of control of diabetes at any given time [30].

In contrast to our findings, another researcher's investigation[31] found no significant relationship between the presence of Candida and the degree of diabetes control, which may be due to differences in sampling techniques or differences in smoking and oral health among patient groups. Additionally, in [32] and Abdullah Ali et al.'s study, they found that the frequency of Candida isolation was significantly higher in smokers and disabled people than in non-smokers in both diabetics and controls. Changes in blood glucose levels can have a rapid and direct impact on the amount of yeast colonization in the mouth cavity.

The growth of the widespread infection depends on the total number of yeast present. To change from a commensal to a pathogenic mode of life, the yeast must aggregate in large enough numbers to accumulate enough enzymes to penetrate the mucous membranes and epidermis [33]. Culture usually yields more accurate clinical data, which are crucial for the clinical diagnosis of oral candidiasis. Most of the people with managed diabetes in this study developed candidiasis, based on exfoliative cytology.

The results for five of the control subjects were negative. These results are consistent with the study described in [34], which discovered that the concentration of Candida albicans in the control group was consistently higher than 10,000 organisms per square centimeter, whereas in the patients with candidiasis, it was never higher than 100 organisms per square centimeter. Grzegocka et al. have shown that quantitative cultures can reliably distinguish between carriers and patients with oral candidiasis [35]. Patients with candidiasis had over 400 colony-forming units per milliliter of saliva, compared to those who carried C. albicans.

The different approaches used in the two studies could account for the higher candida colonization results in this one. Although many other studies used automated analysis methods, the transfer rate for the tongue and buccal samples was calculated manually in this study, averaged, and reported as the transport rate [36]. This finding in this study therefore calls for a more comprehensive analysis with an emphasis on determining cut-off values for colonization in both healthy volunteers and diabetics that could serve as useful clinical indicators and support the diagnosis and treatment of oral candidiasis.

CONCLUSIONS

Oral exfoliative cytology is an effective chairside method for evaluating Candida colonization in diabetics. The results of the study showed that diabetic patients had higher levels of Candida colonization than healthy individuals. If oral Candida colonization in diabetics is identified early enough to prevent the colonization from spreading to other areas of the gastrointestinal tract's mucosal lining, the risk of subsequent dissemination into the circulation may be decreased

However, it is crucial to keep in mind that more research is required in this field, particularly with a larger sample size. This would enable a detailed comparison between the cellular changes associated with type 2 diabetes and those resulting from other diseases. Such comprehensive research will enable us to gain a more sophisticated understanding of the specificity and diagnostic potential of these cellular alterations in the context of various medical disorders.

DECLARATIONS

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Competing Interests

The authors have no competing interests to declare.

Ethical Approval

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent

Not applicable.

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