



## Original Research

# Identification of Candidal Species from the Oral Cavity of Diabetic and Non-Diabetic Groups

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## Abstract

*Diabetes mellitus is a common universal endocrine disorder with decreased host immunity towards infections. In these people the most common opportunistic infection is oral candidiasis. Oral candidiasis is most commonly caused by yeast like fungus Candida albicans. In healthy individuals these microorganisms are believed to be commensals but in diabetic patients, it forms severe colonization, even in the absence of any clinically evident oral candidiasis. This type of subclinical colonization can make them more prone to develop deeper mucosal colonization with further dissemination via blood. In the current study, we aimed to identify and compare the frequency of Candida spp. in the oral cavity of diabetic and non-diabetic groups.*

**Materials and Methods:** Swabs were taken from the mouth of 60 participants and were cultured on Sabouraud dextrose agar (SDA) medium. The study was performed on two groups of diabetic patients (n=30) and nondiabetics (n=30) as the control group. The Candida spp. were identified by culture on CHROM agar Candida medium.

**Results:** The frequency of Candida spp. was higher in diabetic patients compared to non-diabetics. The most frequent Candida spp. in the diabetic patients were Candida albicans (%36.66), C. krusei (%16.66), C. glabrata (%10.00), and C. tropicalis. (%3.33). Likewise, C. albicans was the most frequent species (%26.66) in the non-diabetic individuals. In this study, the results of both methods for identification of the isolates were consistent with each other.

**Conclusion:** Xerostomia and disturbance of physiological factors including pH and glucose can promote overgrowth of Candida flora in the oral cavity. These factors are considered important predisposing factors for oral candidiasis in diabetic patients.

**Keywords:** CHROM agar Candida, Diabetes mellitus, Oral candidiasis.

## Introduction

Diabetes mellitus is a common and global epidemic in the new millennium, which is strongly related to lifestyle and economic change, caused chronic hyperglycemia with impairment of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion and action. The World Health Organization (WHO) has expected an increasing development of diabetes to more than 300 million by the year 2025; particularly, with type 2 diabetes mellitus<sup>(1)</sup>. However, T2DM was known as an adult-onset of diabetes in the past, but it has dramatically increasing more recently in young people and known for about 90% of the global incidence of diabetes and its complications<sup>(2)</sup>.

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of candida species particularly *Candida albicans*<sup>[3]</sup>. Numerous risk factors such as age, gender, nutrition, oral hygiene, smoking, dentures, salivary pH disorder, and xerostomia (dry mouth) make diabetic patients more susceptible to oral candidiasis<sup>[4,5]</sup>. Diabetes mellitus (DM) is the most common endocrine metabolic disorder. Approximately 85-90% of diabetic patients are diagnosed with type 2 diabetes (resulting from insulin resistance); in these patients, salivary dysfunctions such as xerostomia, decreased salivary function, lichen planus, tooth decay, and periodontal diseases are common<sup>[6,7]</sup>. Among the reasons making diabetic patients more susceptible to oral candidiasis are high levels of salivary glucose, low secretion of saliva, impaired chemotaxis, and defect of phagocytosis due to polymorphonuclear leukocyte deficiency. The attachment of *C. albicans* to the crystalline hydroxyapatite produces collagenolytic enzyme, which increases crystal solubility and consumes nitrogen of dentin collagen in DM patients<sup>[8,9,10]</sup>. Due to the upsurge in the level of non-albicans *Candida* species as well as azole-resistant isolates, finding a reliable diagnostic method is necessary for the treatment of *Candida*-related infections. The CHROM agar *Candida* is a chromogenic

culture medium for the isolation and identification of *Candida* species based on colony color<sup>[11,12]</sup>. Herein, we aimed to determine the distribution of *Candida* species in the oral cavity with CHROM agar candida to evaluate and compared the amount of yeasts colonized in the oral cavity of diabetic with nondiabetic individuals. Our results can help with improving patient treatment outcomes.

## Materials and Methods

A case control study was carried out in which samples were selected in a simple random sampling technique which included 30 cases of diabetics and 30 cases of age and gender matched controls (normal individuals). Informed consent from each person was taken and ethical clearance has been obtained for this study. A detailed history from the patient was taken which includes age, sex and duration of diabetes and the blood sugar level. The age groups of the samples were within 40 – 60 years. A thorough intraoral examination was carried out for all patients. People were excluded from the study with the following factors: Patients with clinical lesions of oral candidiasis, patients wearing dentures, edentulous patients, patients with harmful oral habits, recent history of antibiotic therapy, acute and chronic diseases, endocrine disorders immunodeficiency diseases, nutritional deficiency diseases. The study groups consisted of DM patients with fasting serum glucose level of higher than 126 mg/dl and medical records of the glycosylated hemoglobin (HbA1c). Sample collection swabs were taken from the mouth of 60 subjects and were cultured on Sabouraud dextrose agar (SDA) medium. The plates were incubated for 72 h at 25°C. Then, pure colonies were transferred on CHROM agar candida for the isolation and presumptive identification of *Candida* species. The *Candida* isolates were identified after incubation for 48h at 37°C. Light green colony color reveals *C. albicans*, steel blue colonies *C. tropicalis*, large, fuzzy, and rosecolored colonies indicated *C. krusei*<sup>[11]</sup>.

## Results

From the samples of 30 diabetic patients and 30 controls, 20 (66.66%) and 11 (36.66%) were positive for *Candida* species, respectively. Table 1 exhibits identification of *Candida* species by growth on CHROM agar *Candida* medium. Of the 20 isolated *Candida* spp. in DM patients, 11 (36.66%) were identified as *Candida albicans*, whereas *C. dubliniensis*, and *C. parapsilosis* were

not isolated from any of the study groups. In the healthy subjects group, 08 (26.66%) *C. albicans* were identified (Table 2). The current results indicated that colonization of *Candida* in the mouth of diabetic patients was more frequent in comparison with the non-diabetic group. In addition, *C. albicans* was the most prominent species isolated from the oral cavity of both groups.

**Table 1.** Identification of *Candida species* by growth on CHROMagar *Candida* medium

<i>Candida</i> species	Colony color on CHROMagar
<i>C. albicans</i>	Light green(19)
<i>C. tropicalis</i>	Blue(1)
<i>C. krusei</i>	Pink–purple(5)
<i>C. glabrata</i>	Pink with a darker mauve center (2)
<i>C. kefyr</i>	Pink(1)

**Table 2.** Frequency of *Candida* species colonization in the oral cavity of the diabetic patients and controls

<i>Candida</i> spp.	Diabetic patients No.(%)	Healthy subjects No.(%)
<i>C. albicans</i>	11 (36.66%)	08 (26.66%)
<i>C. krusei</i>	03 (10%)	02 (6.66%)
<i>C. glabrata</i>	02 (6.66%)	--
<i>C. tropicalis</i>	01 (3.33%)	--
<i>C. kefyr</i>	--	01 (3.33%)
<i>C. krusei</i> with <i>C. Albicans</i>	02 (6.66%)	--
<i>C. glabrata</i> with <i>C. albicans</i>	01 (3.33%)	--
Total	20 (66.66%)	11 (36.66%)

## Discussion

Poor oral hygiene in diabetic patients may increase the level of *Candida* spp. as part of the oral flora and might affect the superficial and systemic fungal infections compared with healthy individuals<sup>[13-14]</sup>. In oral candidiasis, biofilm formation and overgrowth of *Candida* species are significantly higher in diabetic patients. A combination of host and fungal risk factors such as increased salivary glucose, decreased salivary pH, salivary flow reduction, advancing age, dentures, smoking habits, irritation, and xerostomia facilitate *Candida* spp. colonization<sup>[15]</sup>.

Recently, resistance to antifungal agents has been reported in *Candida* species, especially in strains isolated from immunocompromised patients. Our objective was to compare the presence and colonization of *Candida* spp. in the saliva of diabetic patients and non-diabetic controls. We

found higher incidence of *Candida* infection in diabetic patients. The increased candidal colonization in diabetic patients could be attributed to the promotion of the binding of *Candida* to epithelial cells and reduction of tissue resistance against infection. Likewise, salivary glucose and pH levels are correlated with the increased carriage rate of *Candida* in diabetic patients<sup>[16-18]</sup>. In the present study, diabetic patients aged 40-60 years old with high salivary glucose, low salivary pH, history of xerostomia, and low oral hygiene showed more than 50 colonies of *Candida* in their mouth. Our data revealed that 20 (66.66%) of diabetic patients were found to carry *Candida* spp. in their oral cavity compared with the non-diabetic controls (26.66%), which was in accordance with the findings of the previous studies<sup>[19,20,21]</sup>. Although, among the *Candida* species, *C. albicans* has the

highest frequency in the oral cavity, in the last two decades, the incidence of oral candidiasis with other species such as *C. glabrata* and *C. krusei* that are less sensitive to azole compounds has increased. *C. dubliniensis* is mostly detected in the oral cavity of patients infected with the human immunodeficiency virus (HIV) [22,23]. The differentiation between *C. dubliniensis* and *C. albicans* due to the high degree of phenotypic similarity remains a problem. This species produce a distinctive dark green color on CHROM agar Candida compared to *C. albicans* isolates, which are light green [24,25]. In evaluation of subjects with poorly controlled and well-controlled type 2 diabetes using CHROM agar Candida, Melton et al. reported that *C. Dubliniensis* was not found in oral samples, which were incubated at 42°C [26]. In the current study, *C. dubliniensis* and *C. parapsilosis* were not detected in oral samples by CHROM agar candida.

### Conclusion

The candidal load of oral mucosa in DM patients were found to be significantly higher than those of the control group. In the present study, the patients with high salivary glucose, low salivary pH, history of xerostomia, and low oral hygiene showed more than 50 colonies of *Candida* in their mouth. Our data revealed that 66.66% of diabetic patients were found to carry *Candida* spp. in their oral cavity, and *C. albicans* was the most prominent species. Consideration of the possibility of oral *Candida* infections in DM patients is emphasized for improving patient treatment outcomes and reducing healthcare costs.

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