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Original Article

Microbiology Section

Isolation and Speciation of *Candida* in Type II Diabetic Patients using CHROM Agar: A Microbial Study

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ABSTRACT

Introduction: Newer *Candida* species are now becoming increasingly predominant commensal in the oral cavity.

Aim: Aim of the study was to identify and compare different *Candida* species in the oral cavity of Type II diabetic individuals.

Materials and Methods: The present microbial study was carried out for the duration of three months. Sixty participants were included in the study and divided into two groups of 30 individual each. Group I consisted of patients with Type II diabetes while Group II consisted of healthy individuals without diabetes or any other systemic disease. A total of 3 ml of unstimulated whole saliva was collected from them and centrifuged at 5000 rpm for fives minutes. This pellet was plated onto CHROM agar medium plates and incubated at 37°C for at least 3-4 days. CHROM agar plates were visualized daily at 24 hours, 72 hours and followed up to seven days to check for growth. *Candida* speciation was done by counting the different coloured creamy colonies. Comparison of *Candida* spp. between two groups was done by applying the Student's t-test. A p-value<0.05 was considered as statistically significant.

Results: All the species of *Candida*, namely, *Candida albicans, Candida glabrata, Candida dubliniensis, Candida krusei, Candida parapsilosis* except for *Candida tropicalis* showed a significantly higher (p<0.001) occurrence in the diabetic group compared to the healthy group. The highest identified species is *C. parapsilosis*, second being *C. albicans* in both the groups.

Conclusion: *C. parapsilosis* is now considered as one of the significant causes of *Candida* infection in the oral cavity. This increased virulence will affect the global burden of Candidiasis as few treatment options are available for this new pathogen.

Keywords: Agar medium, *Candida parapsilosis,* Oral cavity, Prevalence

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease mainly manifested in adults and frequently associated with other risk factors. Type II diabetes is caused by a failure of the insulin signaling mechanism in the target cells of the body, so these cells are unable to utilize circulating insulin [1]. Candida species have been frequently isolated from the oral cavities of patients with DM. A great number of reports suggest that C. albicans is the most common species that harbor in the oral mucosa of these sensitive patients in high levels. In addition, the highest rate of colonization occurs in diabetic patients with poor glycemic control [2]. Saliva being a distinctive fluid, considerable interest has been shown in it. Advances in technology have helped to move saliva beyond evaluating oral health characteristics to where it now may be used to measure essential features of overall health [3,4].

The frequency of occurrence of *Candida* infections in diabetic patients has also been recognized for many years. Many reports have assessed the prevalence of *Candida* in the oral cavity of diabetics in association with quantitative estimations by various methods such as imprint and oral rinse cultures [4].

Among all the species of *Candida*, *Candida albicans* has been known to be the most common commensal in the oral cavity. The other *Candida* species include *Candida tropicalis, Candida glabrata, Candida pseudotropicalis, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida stellatoidea, C. glabrata,* and *C. tropicalis*. [5].

Recently, there has been a greater surge in the infectious potential of non-albicans *Candida* species in the oral cavity. Identification of these species has become important because they differ both in their potential to cause the disease as well as in their response to the antifungal agents [6].

CHROM agar for speciation of Candida is a differential culture medium which facilitates the isolation and identification of some clinically important species. The use of this medium offers a rapid, simple, and cost-effective technique [7]. CHROM agar contains specific chromogenic substrates. Microorganisms produce enzymes, which reacts with these substrates and these reactions result in the production of differently coloured colonies on the CHROM agar. These colonies represent different species of Candida based on different biochemical properties [8,9]. This study evaluates the advantages of CHROM agar over conventional method for speciation of Candida isolates. A thorough search of the existing literature revealed very few studies that have isolated the Candida species in diabetic individuals and compared it with nondiabetic individuals. Hence, this study was undertaken to isolate different Candida species in diabetic patients and to identify the most common species other than C. albicans and to compare the prevalence of it in diabetics with that of non-diabetic individuals.

MATERIALS AND METHODS

The present prospective study was conducted at the Department of Oral and Maxillofacial Pathology, K M Shah Dental College and Hospital, Vadodara, India, after taking the ethical approval. The study was carried out for the duration of three months (September 2015 to December 2015). Written consent was taken from the participants before their inclusion in the study.

The subjects were divided into two groups;
Group I: Type II diabetic patients (30 individuals)
Group II: Healthy individuals (30 individuals).

Inclusion Criteria

Group I: Any participant having DM without any other oral lesions fulfilling following criteria were included in the study.

1. Random blood sugar (RBS) ≥200 mg/dl or
2. Fasting blood sugar (FBS) >126 mg/dl.

Group II: Patients who did not have DM or any other systemic illness were included in the Group 2 (Control group).

Exclusion Criteria

The subjects who did not satisfy the above mentioned criteria were excluded from the study.

Participants were made to sit in a well-ventilated room. Demographic details of the participant were recorded in a prescribed proforma, then 5 ml venous blood was drawn from cubital vein and collected in ethylenediamine tetraacetic acid test tube for glycosylated haemoglobin estimation in diabetic patient. Blood sample was transferred to the laboratory. For non-diabetic individuals RBS was performed. Participants were asked to rinse their mouth thoroughly 10 minutes before collection to avoid the collection of food debris and 2-3 ml of unstimulated whole saliva was collected by allowing the participant to drool or gently expectorate into clean, sterile test tube. Immediately after collection, the lid of the test tubes was closed and transferred to laboratory within 30 minutes of collection. These samples were stored at 4°C, until analysis. Participants were instructed not to eat or drink anything for at least one hour before the collection of saliva sample. To control the circadian variation, i.e., time-dependent variation in the biological activity of the body, samples were collected during morning hours. The solution was then concentrated by centrifugation at 5000 rpm for five minutes after this procedure; the pellet which remained at the bottom of the tube and was plated onto CHROM agar medium plates (HiMedia, India) and incubated at 37°C for at least 3-4 days. CHROM agar plates were visualized daily at 24 hours, 72 hours, and followed up to seven days to check for colonial growth. Candida speciation was done based on the different colored creamy colonies appeared on CHROM agar culture media [Table/Fig-1,2]. Total numbers of positive cultures of each species were noted in the proforma [10,11].

STATISTICAL ANALYSIS

The mean value of all the *Candida* species was calculated for the two groups, namely, Type II diabetic group and the healthy group. Statistical analysis for comparison between these two groups was performed by Student's t-test. A $p<0.05$ was considered statistically significant.

RESULTS

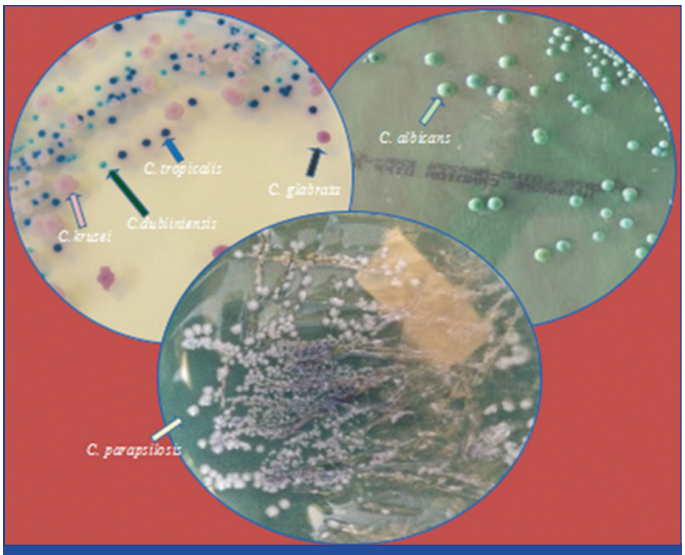
This study included 60 patients, out of which 30 patients had Type II diabetes without any oral lesion, and 30 were healthy individuals. Three samples from the diabetic group were discarded due to contamination. No change in power of the study was seen. All the species of *Candida*, namely, *C. albicans, C. galbrata, C. dubliniensis, C. krusei,* and *C. parapsilosis* except for *C. tropicals* showed a significantly higher occurrence in the diabetic group compared to the healthy group [Table/Fig-3,4].

In this study, the following results were obtained among the diabetic individuals. *C. parapsilosis* (31.22%) had the highest occurrence, while *C. albicans* was found to be 28.01%. The percentages of other species are *C. glabrata* (12.3%), *C. krusei* (11.39%), *C. tropicalis* (5.92%), and *C. dubliniensis* (11.09%) [Table/Fig-5].

In the non-diabetic individuals, *C. parapsilosis* showed the highest percentage of occurrence of 35.87%. *C. albicans* was found to be 25.57%, *C. glabrata* (8.36%), *C. krusei* (7.03%), *C. tropicalis s*(11.39%), and *C. dubliniensis* (11.75%) [Table/Fig-6].

DISCUSSION

DM is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycemia. The normal metabolism of protein, fat, and carbohydrates is affected and impaired. This may be due to either a defect in the secretion of insulin, its action or both *Candida* infections are a major problem in the world, especially among



[Table/Fig-1]: Various types of *Candida* species on CHROM agar plate.

Candida spp.	Colony color on CHROM agar
C. albicans	Light green
C. glabrata	Pale edges on dark pink (purple)
C. tropicalis	Dark blue with halo
C. dubliniensis	Dark green
C. krusei	Fuzzy, rough, large, pink
C. parapsilosis	Cream colored

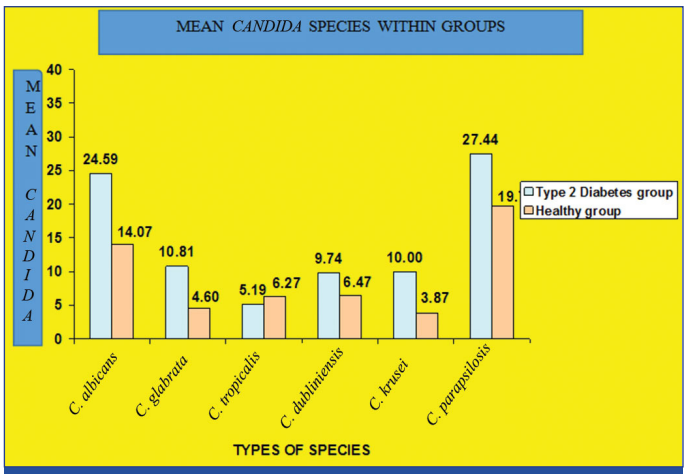
[Table/Fig-2]: Candida species with different colored creamy colonies appear on CHROM agar culture [10,11].

Abbreviations: *C. albicans: Candida albicans, C. glabrata: Candida glabrata, C. krusei: Candida krusei, C. parapsilosis: Candida parapsilosis, C. tropicalis: Candida tropicalis.*

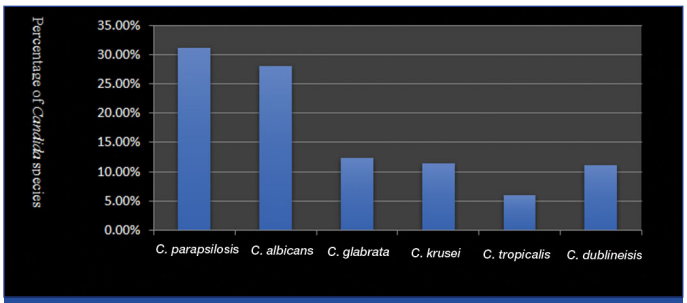
Name of the species of *Candida*	Mean *Candida* species (±SD)		p-value
	Type II diabetes group (N=27)	Healthy group (N=30)	
C. albicans	24.59±07.21	14.07±03.27	0.001*
C. glabrata	10.81±07.77	04.60±04.43	0.001*
C. tropicalis	05.19±03.86	06.27±05.43	0.387 (NS)
C. dubliniensis	09.74±07.05	06.47±03.89	0.037*
C. krusei	10.00±07.50	03.87±02.96	0.001*
C. parapsilosis	27.44±07.33	19.73±07.95	0.001*

[Table/Fig-3]: Comparison of mean *Candida* spp. colonies. By Student's t-test, NS: Not significant, *Significant.

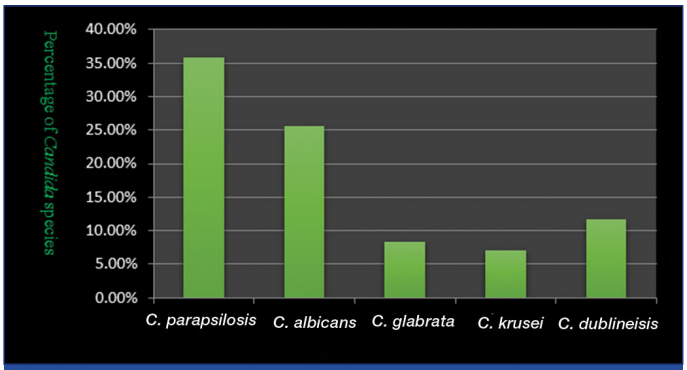
Abbreviations: *C. albicans: Candida albicans, C. glabrata: Candida glabrata, C. dubliniensis: Candida dubliniensis, C. krusei: Candida krusei, C. parapsilosis: Candida parapsilosis, C. tropicalis: Candida tropicalis,* SD: Standard deviation.



[Table/Fig-4]: Comparison of mean *Candida* spp. colonies within groups.



[Table/Fig-5]: Percentage of *Candida* species in Type II diabetic patients.



[Table/Fig-6]: Percentage of *Candida* species in non-diabetic individuals.

the immunosuppressed people. The concomitant occurrence of DM with candidiasis has been established since long, but this association is still being questioned [12].

Compared to the control group, a higher colonization of *Candida* species was seen in the diabetic patients in this study. This finding was found to be statistically significant ($p<0.05$). Mohammadi F et al., in 2006 in their study found that the oral candidiasis frequency in diabetic patients in relation to nondiabetic ones was more due to factors that promote oral *Candida* flora in diabetic patients [13]. Similar results were found by Rosa-García et al., in their study wherein they noted a higher occurrence of *Candida* species in the diabetics than the nondiabetics with *C. albicans* showing the highest occurrence in both the groups [14]. This finding is similar to this study wherein the *Candida* was found to be more in diabetic than the nondiabetic individuals.

In a study by Mohammadi F et al., the frequency of *Candida* species in diabetic group was found to be *C. albicans* (36.2%), *C. krusei* (10.4%), *C. glabrata* (5.1%), and *C. tropicalis* (3.4%), respectively. Likewise, *C. albicans* (27%) *was* the most frequent species in nondiabetic oral individuals [13].

Lydia Rajakumari M et al., in their study, also found the prevalence of *Candida* species in diabetic individuals was higher when compared with nondiabetic healthy individuals. The most predominantly isolated species in diabetic and nondiabetic individuals from buccal cavity was *Candida albicans* [15]. However, these results were not in accordance with our result, wherein *C. parapsilosis* was found to have the highest occurrence both in the diabetic as well in nondiabetic group. Our study suggests that there is a change in pattern of *Candida* spp. found in human oral flora.

LIMITATION

Being a rural dental hospital and dental college, our study had its own limitations such as small sample size, inability to perform antifungal susceptibility tests. Furthermore, three samples from the diabetic group were discarded due to contamination.

CONCLUSION

Candidiasis has been known to be an opportunistic infection since a long time, among which C. albicans being the known the leading cause. However, there has been a shift in this trend. C. parapsilosis has now currently emerged as a human pathogen. Recent advances in diagnostic aids are providing further information about the virulence of C. parapsilosis. Since the morbidity associated with C. parapsilosis is high, it requires urgent attention. Furthermore, there is a dire need to develop targeted antimicrobial agents against it.

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