

# Microscopic Quantification Analysis of Candida in the Oral Cavity of Type II Diabetic Patients compare with Non-Diabetic Human Population

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## ABSTRACT

Since candida inhabits both diseased and normal healthy human population its carriage rate signifies while interpreting the microbiological results associated with candida. In this study oral swabs were collected from the type II diabetic and non-diabetic individuals and the collected specimens were subjected to direct microscopic examination and culture on sabouraud dextrose agar (SDA). The microscopic and culture results had shown difference in these two different study subjects. Compared to non diabetic individuals the oral swab of type II diabetic individuals had shown increased carriage rate of candida. It revealed the facts of the candida colonization among type II diabetic individuals. From this study results it came to understand that the hyper glycemic condition of the type II diabetic patients could be the major reason for the colonization of candida. Candida is a yeast which can attack both immuno compromised and immuno competent humans. Further it needs health care professionals attention to take care of these population.

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## Introduction

Candida is one of the fungi, an yeast inhabits the oral cavity of the human. It also found as normal flora of the vagina. Yeast infections are a particular problem with diabetes because sugar helps candida grow. High levels of sugar in your blood also mean high sugar levels in sweat, saliva, and urine that encourages yeast to grow in places like r mouth and genital parts, and end up with thrush (Michael Dansinger 2022, . Morgan et al 2005) . There are some factors which includes diabetes, hormones, diseased condition of an individual , decreased immune status, immune suppression etc. can favour and facilitate the establishment of candida species which in turn results with candidiasis (Niroshani et al 2006). C.albicans and non albicans species have been proven to be associated with human and animal infections. Since candida emerge as an opportunistic pathogen its presence in human population with and without symptoms should not be ignored. The same time for treatment purpose, the candida presence in the affected human individuals should be correctly diagnosed and interpreted as the causative agent or its unnatural association with such individuals. Hence it is essential to evaluate the significant candida growth among the affected human individuals. The microscopic study of the candida yeast cell numbers its adherence to the buccal as well as vaginal epithelial cells should also be evaluated for interpreting the results before starting anti candidal treatment

Many Research Authors performed studies on the candida colonization among dental carries (Yang et al 2022, Freitas et al 2014, Ko-Adams et al 2020, Naranjo, et al 2006,

Reichardt et al 2019 ). In this study, all the type II diabetic were selectively included those who were all free of dental carries.

### Materials and methods

Totally 1000 humans study population which includes type II diabetic patients (n=500) and non-diabetic healthy human controls (n=500) were selectively included in this study, all individuals were free of dental carries and other types of oral infections .both males and females belongs to the age group 35-65 were included in this study .

### Specimen collection

Early morning specimen collection was preferred. All human subjects were thoroughly taught and trained by the qualified doctors ( for how to collect oral swab ). Two swabs were collected from each individuals and processed for microbiological evaluation. Among two swabs, one was placed in a tube containing 0.5ml sterile distilled water and that was sent to the laboratory for further proceedings. Just before culture on SDA , in order to homogenize the collected specimens, the swab in test tube with distilled water was gently shaken for 5 minutes on rotator. From the second swab a smear on glass slide was made. To obtain better results, a drop of normal saline was placed on the glass slide and smear was made from the swab.

### Microscopic quantification of candida

The method of B V Naidu and B A Reginald 2016 used for candida quantification was used in this study with height modifications. The smear made from the oral swab was subjected to gram stain. The number of budding yeast cells with or without pseudo hyphae, buccal epithelial cells and its adherence with yeast cells, were microscopically examined .

The microscopic candida quantification was evaluated as following 0/-negative, 0-10/ moderate, >10/100x – significant and uncountable-crowd- colonization the results of control and test group was recorded and compared.

### Results and discussion

Candida species associated with human infections and it attracts the interest of the scientists and researchers at global level. Diabetes Mellitus (DM) is one of the non infectious diseases, a metabolic disorder which predisposes the human to different types of microbial infections. The immune suppressive status of the diabetic population favours candidiasis (Celia et al 2019, Karaa A., Goldstein, 2015 ).

The association between the diabetic individuals and candida had been extensively studied by many authors (Belazi et al 2005, Darwazh et al at 1990, Goncalves et al 2006 and Gudlaugsson et al 2003).even the earlier research 19th century, the Davenport et al 1970, studied candida oral distribution among denture stomatitis.

In the year 2002, the author Kadir et al, performed study on candida carriage in diabetic and non diabetic human individuals. It has been already noticed that the increased level of serum glucose concentration among the DM patients than the non diabetic healthy individuals . The hyper glycemc condition of the saliva associated with higher oral candida carriage in diabetic individuals. A rapid saliva PH decline was noticed by the authors and that was justified reason with the over growth of candida (Preethi et al 2016)

Our study correlates with the above mentioned statements all study population which includes both DM patients and normal healthy non DM individual's saliva was checked for PH. The increased PH level was recorded with DM population. The maximum PH of the study groups was recorded as 4.5 and 8.5 in Type II DM patients and non diabetic healthy human individuals respectively. Torres et al 2003 studies the clinical aspects of candida carriage among xerostomic individuals and documented the low salivary flow in the oral cavity predispose the candida establishment. The same point had been recorded in our study . Most of the type II diabetic patients of our study group found to be recorded with dry mouth due to decreased salivary flow.

The micro biological analysis of the oral swab specimens collected from the Type II diabetic and non diabetic individuals had shown significance in their results. The number of yeast cells and its adherent pattern to those two

groups of humans shown to have difference. Overall the number of candida yeast cell presence in the oral swab specimens of Type II diabetic patient has shown increased numbers while the non diabetic human population specimen showed comparatively decreased number of candida yeast cells. From this it came to understand that the hyper glycemc condition of the Type II diabetic patients could have been associated factor which may be the probable facilitating factor for the colonisation of the candida yeast cells. The maximum number mean of candida yeast cell presence per high power field of highest microscope was recorded as 53/100x and 11/100x for Type II diabetic and non diabetic individuals respectively.

### Candida adherence

When candida present in host as normal flora usually it resides in the mucus layer of mucosal surfaces. However rarely it may become as opportunistic pathogen under favourable conditions and directly encounter the host cell. The initial step of the establishment of candida is the attachment to the epithelial cells. Further the favouring conditions such as hormonal imbalance, decreased immunity, malnutrition, in case of diabetic patients the hyperglycemc condition etc facilitates its multiplication finally result in candidiasis. It was extensively studied by many authors and acknowledged by the researchers at global level. The predisposing factors for candidiasis can be subdivided into immunologic and non immunologic factors according to occurrence ( (Shweta et al 2015).

The microscopic quantification of candida adherence to the buccal epithelial cells of the oral swab collected from Type II diabetic patients and non diabetic healthy humans had shown increased and decreased yeast cell adherence respectively. The maximum number of candida yeast cell adherence to the buccal epithelial cells of oral swab specimen of the Type II diabetic and non diabetic study population was noticed as 18/BECs respectively.

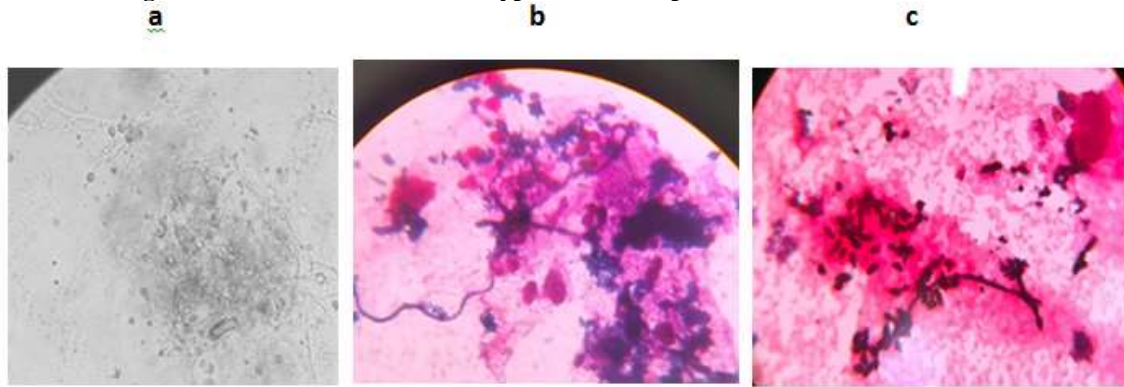
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**Table 1. Microscopic quantification of oral swab collected from study population**

S. No	Study population	No.of .candida			Candida adherence on BECs		
		<10	>10	Crowd	<10	>10	Heavy adherence
1.	Type II Diabetic patients N=500	11	274	315	21	148	431
2.	Non-diabetic healthy individuals (Voluntary n=500)	371	86	43	328	149	03

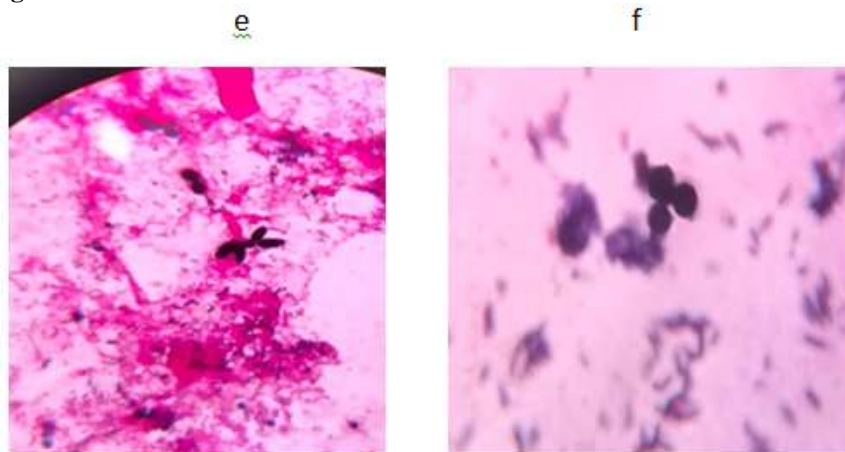
**Note;-BECs – Buccal Epithelial Cells - PQ value – statistically significant**

**Fig 1. Oral swab collected from Type II diabetic patients - number of candida**

a). 10% KOH wet mount showing buccal epithelial cells adhered with many yeast cells and pseudohyphae.

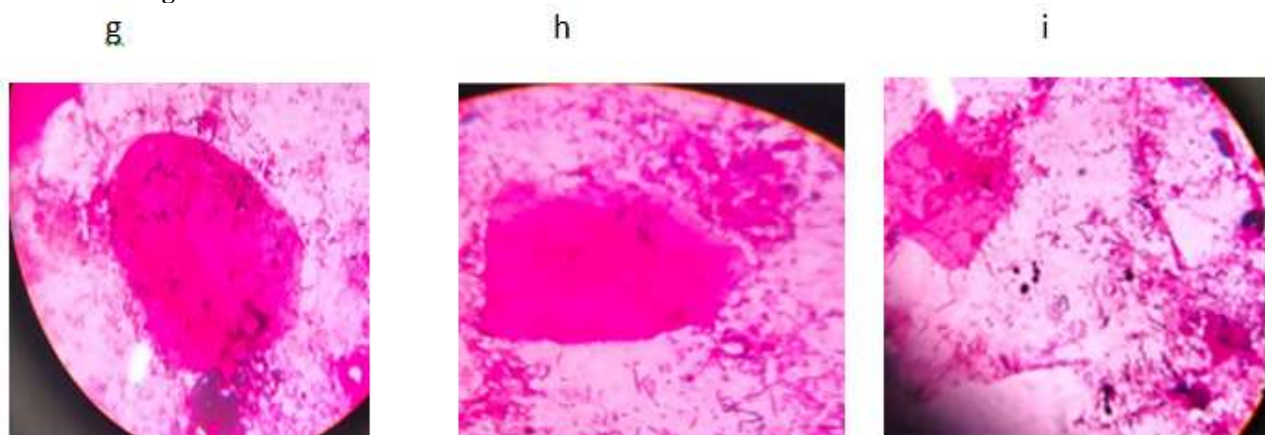
b). Oral swab from sugar controlled diabetic case - gram stained smear presenting with more than 10 gram positive budding yeasts with pseudohyphae morphologically suggestive of candida. C) oral swab from sugar uncontrolled diabetic case - presence of many yeast cells with pseudohyphae and other bacterial microbiota with biofilm ( pink colored filmy mass around gram negative bacterial population)

Calderone, et al 1991 and Silva, et al 1995 reported about the germ tube formation and pseudohyphae production among candida species and their association in the attachment of candida to the different surfaces including BECs and Vaginal ECs. In our study it has been observed that the presence of increased pseudohyphae attached to the BECs of the type II diabetic patients, which implies not only the hyperglycemic condition but also the phenotypic switching from yeast to hyphal form of candida also play an important role in its adhesion ( fig. 1. b & c).

**Fig 1A. Oral swab from non diabetic normal individuals - number of candida**

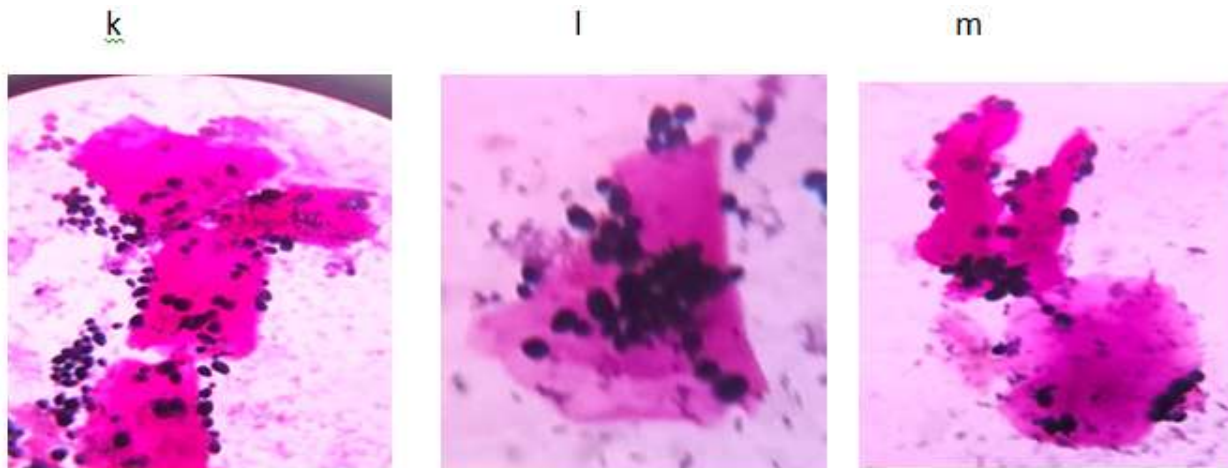
e) & f) -note countable number ( less than 10 candida cells) of candida suggestive of its presence as commensals .

A decreased flow of saliva rate found to be the one of the major causes for the colonisation of Candida in oral cavity ( Torres et al 2003 and Buranarom et al 2020). Xerostomia is one of the symptoms of the diabetic patients. In this study all type II diabetic patients compliance with decreased saliva flow and dryness of the oral cavity and their oral swab microscopical analysis had shown increased number of candida and candida adherence to the BECs. The reason could be the hydrophobic nature of the oral cavity and the BECs which may enhances the adherence.

**Fig 2. Oral swab from non diabetic normal individuals - candida adherence on BECs**

h) & h) – BECs- buccal epithelial cells without candida . i)- budding candida yeast cell present but not adhered on BECs

**Fig 3A. Oral swab from type II diabetic normal individuals - candida adherence on BECs**



k) , l & m) – showing numerous candida yeast cells adhered on buccal epithelial cells – note destroyed buccal epithelial cells – ( fig.m)

Mucin in the saliva and the oral mucosa together acting as the protective shield thereby put a stop to the adherence of microbiota in the oral cavity ( Nikou et al 2019 ) . These natural protective mechanism is oppressed by the diabetes and the related signs and symptoms, mainly the hyper glycemc condition and the xerostomia among the diabetic population.

### Conclusion

Candida is a yeast harbouring the oral cavity of both immunocompetent and immune compromised hosts. The number of candida present in the oral cavity of the type II diabetic and non diabetic is varied. From this study it has been concluded that the oral swab specimens found to be carrying increased number of candida yeasts while the swab specimens of non diabetic healthy individuals found to shown decreased number. Candida yeasts not adhered to the BECs of the non diabetic individuals were identified, if adhered that too less than ten yeast cells. In case of type II diabetic patients BECs were heavily adhered with candida. The main reason for this findings could be due to the hyperglycemic condition of the diabetic population because fungi are sugar lovers. However the heavy load of candida yeasts presence in the oral cavity may facilitate further colonisation of candida which may result in candidiasis among these type II diabetic patients. From this, it is suggested that the periodical check up for candida quatification is essential for these risky group to avoid such complication.

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