

## Identification of *Candida albicans* in Oral Candidiasis by Germ Tube Test

Anshi Jain<sup>1</sup>, Madhumita Barua<sup>2</sup>, Kiran Kumar<sup>3</sup>, Devi Charan Shetty<sup>4</sup>

**Background:** Oral candidiasis is caused by overgrowth or infection of oral cavity by yeast like fungus, *Candida*. Out of pathogenic 20 species, *C. albicans* is most common species associated with oral candidiasis. Oral candidiasis marks systemic disease such as diabetes mellitus and commonly encountered among the immunocompromised patients with HIV as opportunistic infections. It appears as white or cream colour *patches* and often bleeds if rubbed or scraped. Though the diagnosis of oral candidiasis can be made clinically, laboratory procedures and mycological cultures have to be used for the confirmation of the diagnosis. **Aims:** To assess the association of *C. albicans* species with Oral candidiasis using Germ Tube Test. **Method and result analysis:** The study was conducted in Department of Oral Pathology and Microbiology and Department of Oral Medicine and Radiology ITS-CDSR. A total of 25 patients clinically suggestive of oral candidiasis were selected. After obtaining consent, oral scrapings were collected and subjected to see the presence of fungal element using 10% KOH. Further the samples were inoculated on Sabouraud Dextrose Agar and Blood Agar for fungal isolation and identification of *Candida albicans* using Germ Tube Test. **Results:** The number of cases associated with *C. albicans* were positive for germ tube test and analysed statistically

**Key words:** Candidiasis, Germ Tube Test, Sabouraud Dextrose Agar, 10% KOH.

### INTRODUCTION

*Candida albicans* is a dimorphic fungus that exists as a commensal of warm-blooded animals including humans. It colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract and is also able to cause a variety of infections, depending on the nature of the underlying host defect. Therefore, *C. albicans* infections (candidiasis) are very infrequent in healthy individuals. Candidiasis may be divided into superficial

(such as oral and vaginal thrush and chronic mucocutaneous candidiasis) and deep-seated (such as *Candida* due to myocarditis and acute disseminated *Candida* septicaemia) and represent a major clinical problem. For several reasons (immunosuppressive treatments, long-term catheterization, use of broad-spectrum antibiotics and longer survival of immunologically compromised individuals), *Candida* infections have increased dramatically over the last two decades.<sup>1</sup>

**Corresponding Author :** Dr. Anshi Jain, Post graduate student, Department of Oral and Maxillofacial Pathology and Microbiology I.T.S. Center for Dental Studies and Research, Muradnagar, Ghaziabad (U.P) - 201206, India. (M) 09971967560

<sup>1</sup> Post Graduate Student, Department of Oral and Maxillofacial Pathology and Microbiology, I.T.S. Center for Dental Studies and Research, Muradnagar, Ghaziabad (U.P) - 201206, India.

<sup>2</sup> Reader, Department of Microbiology, I.T.S. Center for Dental Studies and Research, Muradnagar, Ghaziabad (U.P) - 201206, India.

<sup>3</sup> Associate professor, Department of Oral and Maxillofacial Pathology and Microbiology, I.T.S. Center for Dental Studies and Research, Muradnagar, Ghaziabad (U.P) - 201206, India.

<sup>4</sup> Professor and HOD, Department of Oral and Maxillofacial Pathology and Microbiology, I.T.S. Center for Dental Studies and Research, Muradnagar, Ghaziabad (U.P) - 201206, India.

An interesting feature of *C. albicans* is its ability to grow in two different ways: yeast cells (also known as blastospores), Pseudohyphae cells and true hyphal cells. Yeast cells are round to ovoid in shape and separate readily from each other. Pseudohyphae resemble elongated, ellipsoid yeast cells that remain attached to one another at the constricted septation site and usually grow in a branching pattern that is thought to facilitate foraging for nutrients away from the parental cell and colony. True hyphal cells are long and highly polarized, with parallel sides and no obvious constrictions between cells. Actin is always localized at the tip of the growing hypha. Transitions between the two phenotypes can be induced in vitro in response to several environmental cues such as pH or temperature, or different compounds such as N-acetylglucosamine or proline. However, perhaps the most critical criterion for pathogenicity is the induction of the mycelial form by serum or macrophages. In addition to the intrinsic biological interest of this dimorphism, its ability to switch between the yeast and the hyphal mode of growth has been implicated in its pathogenicity.<sup>2</sup>

The germ tube (GT) is a characteristic morphology observed only in *C. albicans*, confirmation of GT is available as a rapid method for identifying *C. albicans*. GT formation was first reported by Reynolds and Braude (1956). They observed yeast cells transforming into filamentation (germ) tubes in human blood, sera, plasma and cerebrospinal fluids. Since then, many substances were reported as GT inducers, such as serum. Germ tube test is considered as rapid & accurate method available for identifying *Candida albicans*. It is 87.1% sensitive and 100% specific for the identification of *C.*

*albicans*.<sup>3</sup>

The present study was undertaken to determine the presence of *C. albicans* species in Oral candidiasis by using Germ Tube Test.

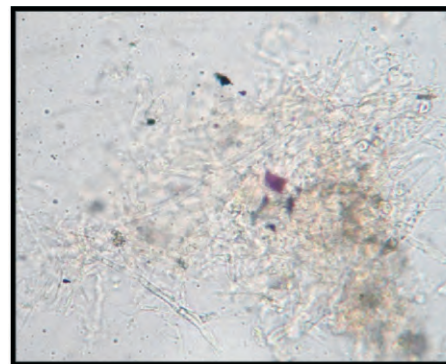
#### MATERIALS & METHODS

A total of 25 patients clinically suggestive of candidiasis were selected. After obtaining consent, oral scrapings were collected and *C. albicans* were identified using a series of laboratory investigations.

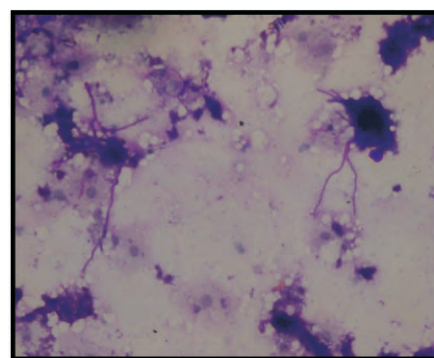
The following protocol was employed to confirm the diagnosis of the suspected cases of different form of candidiasis.

##### ❖ Direct examination

The clinical specimens were collected from the patients with the help of sterile swabs or scraping. These were examined in the 10% KOH wet mount (fig: 1) and by performing PAS staining (fig: 2).



*Fig:1 10% KOH (10X)*



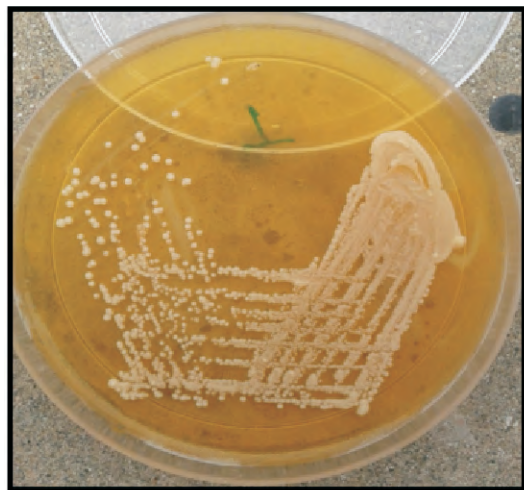
*Fig:2 PAS staining (40X)*

**Fungal culture**

The clinical specimens were cultured on Sabouraud dextrose agar with antibacterial antibiotics and incubated at 25°C and 37°C. The colonies appeared in 3-4 days as cream colored, smooth and pasty. ( fig3 and 4)

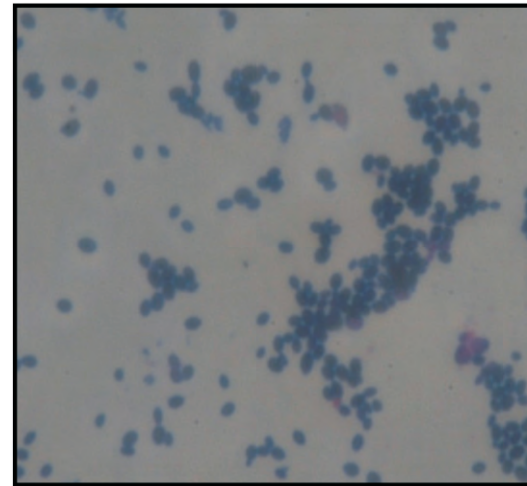


*Fig3: Blood agar culture*



*Fig4: Sabouraud dextrose agar*

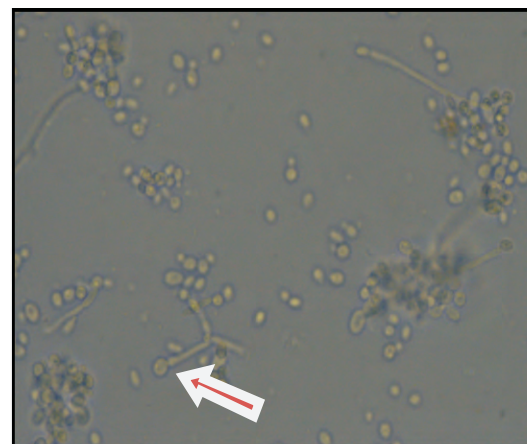
Gram staining was performed by taking sample from culture media to appreciate the presence of candidal colony. (Fig 5)



*Fig: 5 Gram Staining (40X)*

❖ Germ tube test

The culture of candida species was treated with pooled normal human serum and incubated at 37°C for 2 to 4 hours. Wet mount was prepared and visualized under 40X. The germ tubes were seen as long tube-like projections extending from the yeast cells. ( fig: 6)



*Fig 6: Germ tube test (40X)*



## RESULTS

**Table 1: Rate of isolation of Candida from clinical samples**

Number of patients	Candida isolated	Candida negative
25	19 (76%)	06 (24%)

❖ On the basis of SDA and blood agar culture

**Table 2: %Distribution of Germ Tube Test**

Candida Isolated	Germ tube test positive	Germ tube test negative
19	15 (79%)	04 (21%)

❖ On the basis of germ tube test

Among all the patients with candidal infection, 79% were proved to be associated with *Candida albicans* which is proved by germ tube test.

## DISCUSSION

*C. albicans* is a dimorphic fungus that exists as a commensal of warm-blooded animals including humans. *Virulence of Candida albicans* is associated with the ability to switch between the yeast and hyphal morphologies. *C. albicans* grows vegetatively in at least three morphogenic forms: yeast, pseudohyphae and hyphae. The yeast form closely resembles the budding yeast *S. cerevisiae*. The pseudohyphal form consists of chains of elongated yeast cells that retain constrictions at the junctions between adjacent compartments, whereas hyphae are tube-like, with sides that are parallel along their entire length. Pseudohyphae can sometimes superficially resemble hyphae; however, the both states are clearly different and should not be confused. The term filamentous is used here where it not clear whether cells are

hyphal or pseudohyphal. The *C. albicans* hyphal form is often found at sites of tissue invasion, and cells that do not readily form hyphae often have reduced virulence. Importantly, other *Candida* species that do not readily form true hyphae are much less frequently isolated from the human host, indicating that they are less virulent. But strains that are unable to grow in the yeast form are also less virulent. It is generally thought that hyphal cells expressing cell-wall proteins that facilitate adhesion to human tissues are important for tissue invasion, as well as for escape from phagocytosis mediated by neutrophils or macrophages. By contrast, the yeast form is thought to be important for dissemination of the pathogen through the blood stream. It is likely, therefore, that the ability to switch between the morphological forms is important for *C. albicans* virulence. Studies have shown

the incidence of *C. albicans* associated with oral candidiasis is 60-75%. Other species of candida which are frequently isolated from oral candidiasis are *C. tropicalis*, *C. glabrata*, *C. pseudotropicalis*, *C. guilliermondii* and *C. krusei*.<sup>4,5</sup>

Though the diagnosis of oral candidiasis can be made clinically, laboratory procedures and mycological cultures have to be used for the confirmation of the diagnosis. Solely on the basis of Sabouraud Dextrose Agar culture *Candida albicans* cannot be a confirmatory diagnosis. Formation of Germ Tubes is a characteristic morphology observed in *Candida albicans*. It is a cylindrical outgrowth initiated on the surface of blastospore in the presence of environmental factors. There is no constriction at the point of attachment to yeast cell as seen in case of Pseudohyphae. Confirmation of Germ Tube is available as a rapid method for identifying *C. albicans*.<sup>6,7</sup>

Our study showed that 79% cases of candidal growth are positive for germ tube test.

#### CONCLUSION

Hence it is concluded that all clinically looking candidal infection are not actually caused by candida. They have to be investigated for the causative agent as this study shows that out of total 25 patients only 19 are associated with candidal infection. With the help of germ tube test it was proved that only 75% of patient with candidal infection are associated with

*Candida albicans* thus confirming that even other species of candida are also present in oral candidiasis.

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