

## ORAL CANDIDAL CARRIAGE IN SUBGINGIVAL SITES AND ITS SUBSPECIES IDENTIFICATION IN DIABETIC AND NON-DIABETIC PATIENTS WITH PERIODONTITIS

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### Abstract

Candida is the most common fungal pathogen of oral cavity in humans. It exists as a commensal inhabitant of mucosal surfaces in most healthy individuals. Periodontitis is an inflammatory disease of the periodontium with multifactorial etiology. There is a positive relationship between periodontal inflammation and type 2 diabetes. Diabetes is a metabolic disease characterized by hyperglycemia due to defects in insulin production, insulin action, or both. Diabetes mellitus can impair the function of polymorphonuclear leukocytes which may predispose diabetic patients to greater risk of diseases including periodontal disease and oral candidal infections.

The objective of this study is to evaluate the oral candidal carriage in the subgingival sites and also to evaluate the predominant candidal subtypes in non-diabetic individuals with periodontitis, diabetic individuals with periodontitis and healthy individuals without periodontitis.

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### Introduction

Diabetes is a most common metabolic disorder characterized by hyperglycemia due to defects in insulin production, insulin action, or both<sup>1</sup>. Periodontitis is the most common inflammatory disease of the oral cavity. Emerging research suggests that the relationship between the two is not coincidental but causative. Not only are people with diabetes more susceptible to periodontitis, but, periodontal disease may have the potential to affect blood glucose control and contribute to the progression of diabetes.

People with diabetes are at an increased risk for periodontal disease because they are generally more susceptible to bacterial infections and have a decreased ability to fight bacteria that invade the gingiva.

Candida albicans express virulence factors that may have an important role to the pathogenesis of periodontal disease, such as the ability of penetrating the epithelium, inhibiting PMN cells and causing lysis of monocytes<sup>2</sup>. The link between periodontal and systemic health is two-way, particularly when it comes to periodontitis and diabetes mellitus<sup>3</sup>.

The objectives of this study include:

To evaluate the prevalence of oral candidal carriage in subgingival sites and its subspecies identification in:

- Diabetic individuals with periodontitis
- Non-Diabetic individuals with periodontitis
- Healthy individuals without periodontitis.

To compare the prevalence of oral candidal carriage in GCF across the 3 groups.

To compare and contrast different candidal subspecies in GCF across the 3 groups.

### Materials and methods

This study was carried out in the Department of Oral Pathology & Microbiology, Sharad Pawar Dental College (DMIMS), Sawangi (Meghe), Wardha.

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**Subject:** The study comprised of,

**Group 1:** 30 diagnosed cases of type 2 diabetes mellitus patients with periodontitis were selected from the Department of Periodontia, SPDC, Sawangi (Meghe), Wardha.

**Group 2:** 30 non-diabetic individuals with periodontitis were selected from the out patient department of SPDC, Sawangi (Meghe), Wardha.

**Group 3:** 30 healthy individuals without periodontitis were selected from the out patient department of SPDC, Sawangi (Meghe), Wardha.

**Sample collection:**

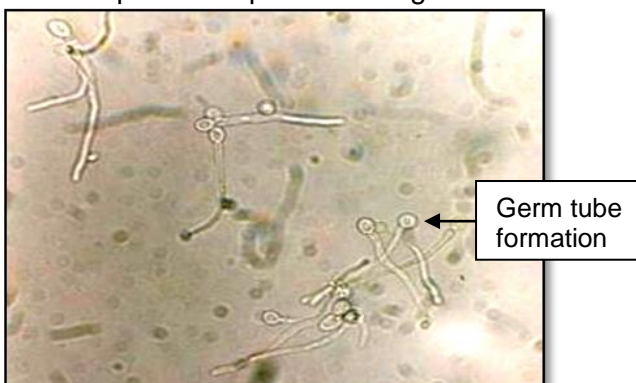
For evaluation of candidal carriage in subgingival sites, gingival crevicular fluid (GCF) was collected by inserting the absorbent paper points in the gingival sulcus and was subjected to culture.

**Culture:**

The absorbent paper points with GCF sample of all the patients were immediately inoculated on Sabouraud's Dextrose Agar (SDA, Himedia-India). For each patient, 2 SDA were inoculated and the media were incubated at 37° C. Within 24-48 hrs. at 37°C, growth on SDA was identified as Candida species.

**Germ tube test (Reynolds - Braude phenomenon)**

This rapid screening procedure for observing germ tube formation identifies and differentiates *C. albicans* from other Candida species. The culture of Candida species was treated with 1 ml of sterile mammalian (fetal - bovine, sheep or normal human) serum and incubated at 37°C for 2 to 4 hrs. After incubation, a drop of suspension was examined on the glass slide under the microscope for the presence of germ tube.



**Figure 1.** Photomicrograph of germ tube formation.

**KB006 Hi Candida Identification Kit**

This kit is a standardized colorimetric identification system utilizing 12 conventional biochemical tests. It is based on the principle of pH change and substrate utilization. Each well was inoculated with 50 µl of the organism suspension by surface inoculation method and incubated at 22.5°C ± 2.5°C for 24- 48 hrs.



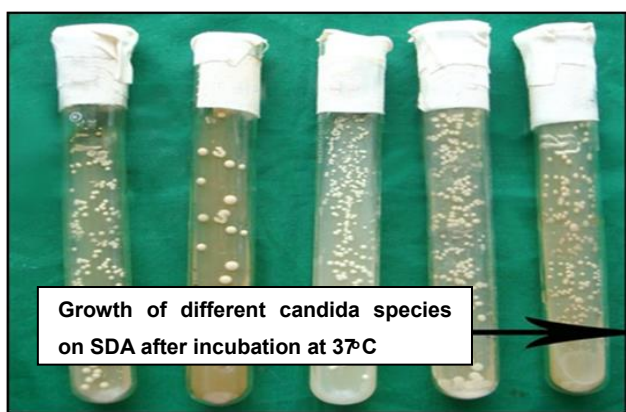
**Figure 2.** KB006 Hi Candida Identification Kit.

**Results**

The colony morphology interpretation was as follows:

| MORPHOLOGY  | CANDIDA SPECIES      |
|---|----------------------|
| Cream colored, pasty and smooth   | Candida albicans     |
| Cream colored to off -white, glistening to dull, soft, smooth or wrinkled with mycelia fringe | Candida tropicalis   |
| Cream colored, flat, dull and dry   | Candida krusei       |
| Cream colored, glistening, smooth   | Candida glabrata     |
| Cream colored, smooth and pasty   | Candida dubliniensis |

**Table 1.** Colony morphology interpretation.



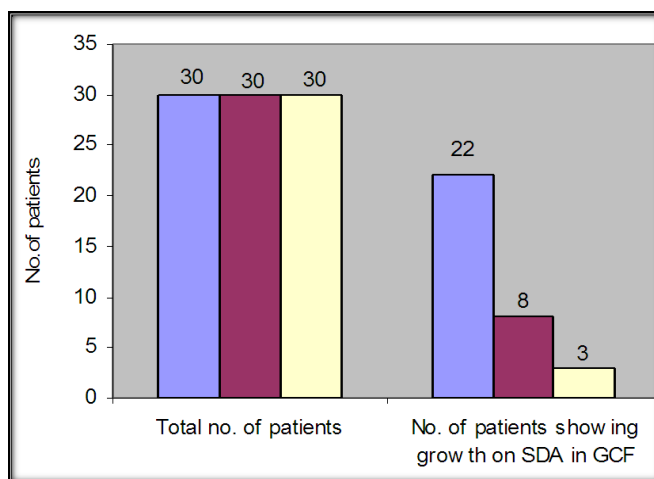
**Figure 3.** Growth of different candida species on SDA.

**Number of patients showing growth on SDA:**

|              | Total no. of patients | No. of patients showing growth on SDA in GCF |
|--------------|-----------------------|--|
| Group 1      | 30                    | 22   |
| Group 2      | 30                    | 8  |
| Group 3      | 30                    | 3  |
| z-value      |                       |  |
| Group 1 Vs 2 |                       | 4.08, Significant                            |
| Group 1 Vs 3 |                       | 6.49, Significant                            |
| Group 2 Vs 3 |                       | 1.70, Non Significant                        |

**Table 2.** Number of patients showing growth on SDA

**GRAPHICAL REPRESENTATION SDA**



■ Group 1 ■ Group 2 ■ Group 3

**Figure 4.** Graphical representation of no. of patients showing growth on SDA.

Identification of Candida species using KB006 Hi Candida Identification Kit in all the 3 groups in GCF.

| SPECIES              | GROUP 1 | GROUP 2 | GROUP 3 |
|----------------------|---------|---------|---------|
| Candida albicans     | 13      | 6       | 3       |
| Candida dubliniensis | 9       | 2       | 0       |
| Total                | 22      | 8       | 3       |

**Table 3.** Identification of Candida species using KB006 Hi Candida Identification Kit.  
**C.albicans >> C.dubliniensis**

**Discussion**

**Gingival Crevicular Fluid (GCF)**

Gingival crevicular fluid is a fluid occurring in minute amounts in the gingival crevice. The presence of sulcular fluid helps in regular maintenance of Periodontium and plays an important role in oral defense mechanism which is hampered in diabetes.

**Alterations in Gingival Crevicular Fluid (GCF) in diabetes:**

The various alterations in gingival crevicular fluid (GCF) in diabetes include

increased glucose level in GCF, impaired function of PMNs, increased collagenase activity, increased accumulated glycation end products (AGEs)<sup>4</sup>. Collagen is cross-linked by AGE formation, making it less soluble and less likely to be normally repaired or replaced. As a result, collagen in the tissues of patients with poorly controlled diabetes is aged and more susceptible to breakdown. Also, there is increase in pro-inflammatory mediators like Interleukin-1 $\alpha$ , 1 $\beta$ , TNF-  $\alpha$ , PGE2 and increase in the altered GCF flow<sup>5</sup>.

### Increased prevalence of candida in GCF of diabetic patients

The increased glucose level in GCF in diabetic patients could change the environment of microflora, inducing qualitative changes that could contribute to the susceptibility and severity of infections.

The deficiency of PMNs may result in impaired chemotaxis, defective phagocytosis or impaired adherence leading to increased susceptibility of diabetic patients to infection resulting in immune dysregulation.

A drop in pH from 7 to 3 in diabetes makes the GCF acidic, which in turn favors the growth of candida<sup>6</sup>.

### GCF favors the growth of *C.albicans* & *C.dubliniensis* - more pathogenic candida species

The transition from a commensal to a harmful pathogenic state eventually depends on decrease in host resistance, changes in the local environment and changes in the intrinsic fungal virulence.

### Various virulence factors of candida species which helps in the progression of periodontal disease in diabetes:

**Adherence:** The increase in adherence of candida species to the oral mucosa depends on specific interactions of lectin-like proteins of the fungal cell wall with terminal sugars of the cell surface glycoproteins of the human host<sup>7</sup>.

**Invasion:** Another virulent attribute of candida is its ability to invade the superficial layers of epithelium. Hyphal appendages and certain candidal proteinases (aspartyl proteinase) and lipases are known to promote candidal invasion. They act as keratinase and hence may be

involved in fungal invasion through keratinized mucosa<sup>8</sup>.

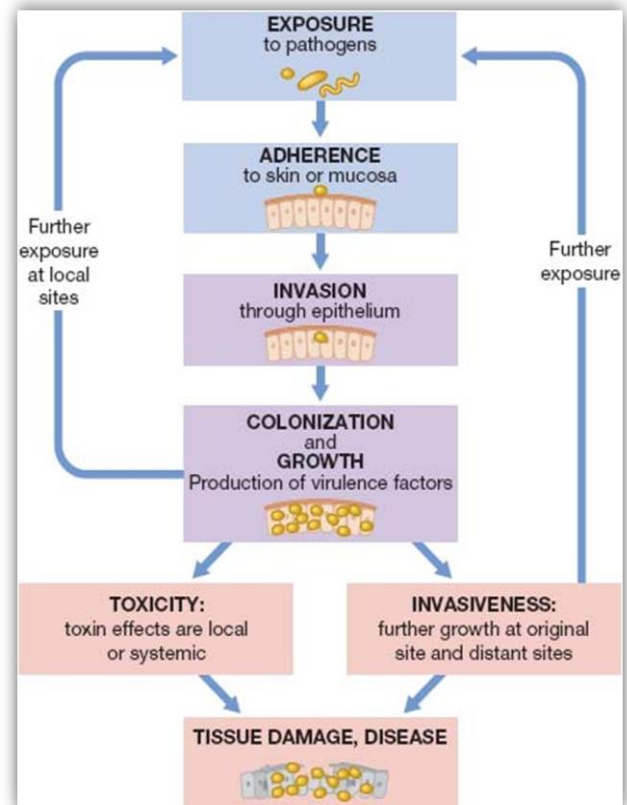


Figure 5. Virulence factors of candida species.

### Interference with phagocytosis

The first line of the host defence against candidal invasion consists of phagocytosis, with polymorphonuclear neutrophils being of foremost importance. Certain isolates of *C. albicans* produce acidic peptides which inhibit the attachment of fungal hyphae to phagocytes<sup>9</sup>.

### Inhibition of immune system

Inhibition of parts of the immune defences of the host by *C. albicans* has been observed. There is candida dependent proliferation of suppressor cells of the B- lymphocyte lineage<sup>10,11</sup>. Also, a polysaccharide fraction of *C. albicans* has been shown to inhibit the proliferation of human T- lymphocytes and the production of interleukins 1 and 2<sup>12</sup>.

### Conclusions

Gingival crevicular fluid helps in proper maintenance of periodontium, however in diabetes, this positive correlation becomes

negative as GCF leads to its damage by harboring the pathogenic candida.

Some *Candida* species are normally present in oral cavity as commensals, but in diabetes, there is transition from commensal to pathogenic state.

### Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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