

## Isolation of *Candida albicans* from oral cavity of type II diabetic subjects and its relationship to total and differential white blood cell count

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### Abstract

**Background and objective:** It is well known that oral candidiasis increase in many situations, like obesity, debility, leukemia, viral infection, use of certain drugs in addition to diabetes mellitus. The aim of this study was to estimate the prevalence of *Candida albicans* in the oral cavity of diabetic and non-diabetic subjects and to identify factors predisposing to colonization in the diabetic patient. The variables evaluated include absolute white blood cell counts and differentials, glycosylated hemoglobin levels, serum glucose, blood urea, serum creatinine and duration of diabetes.

**Methods:** One hundred subjects of type II diabetes mellitus and one hundred non-diabetic subjects (control) were studied for isolation of *Candida albicans* from oral cavity. Further investigations for diabetic group were done regarding serum glucose, HbA1c, and total and differential white blood cell counts.

**Results:** This study showed 56 (56%) out of 100 diabetic subjects and 30 (30%) out of 100 in non-diabetic subjects were found to carry *Candida* in their oral cavity. In the diabetic group, no relationship was found to total or differential white blood cell count, recent use of antibiotics, serum glucose and HbA1c values. A significant relationship was found in diabetic patients who had chronic renal disease.

**Conclusion:** Colonization of *Candida albicans* in the oral cavity was found to be higher in diabetic subjects than in non-diabetic. However, glycaemic control in diabetes, total and differential white blood cells were found to bear no relation with carriage of *Candida* in the oral cavity.

**Keywords:** *Candida albicans*, type II diabetes mellitus, white blood cells.

### Introduction

Candidiasis is an infection caused by any of several species of the fungus *Candida*. By culture, *Candida* is considered normal oral flora at a frequency of 44-55%.<sup>1</sup> This observation is supported by well controlled studies with exfoliative cytology.<sup>1,2</sup> Although numerous candidal species can be isolated from the oral cavity, the predominant species affecting hospitalized patients is *Candida albicans*.<sup>3-5</sup> Clinical diagnosis of oral candidiasis relies on the recognition of granular, erosive, and pseudomembranous forms of the infection, with the easily removed curd like plaques of the latter being the most common.<sup>6</sup> However, substantial colonization can exist in the

absence of clinical lesions.<sup>2</sup> Although cultures have been favored for confirmation of clinical infections, periodic acid-Schiff-stained cytologic smears are also an excellent method; they hold a marked time advantage over and are less costly than cultures.<sup>1</sup> In fact, cytology offers a further advantage in facilitating a distinction on morphological grounds between a carrier state and active infection. Because the pseudohyphal phase is considered the invasive phase of the fungus, the diagnosis of mucosal candidiasis relies on the demonstration of these forms, as well as blastospores.<sup>6</sup> The high carrier rate of *Candida* in a normal population emphasizes the advantage of cytology because it

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has been stated that positive cultures by themselves are inadequate for the diagnosis of oral candidiasis.<sup>7</sup> Groups classically considered at increased risk for candidal infections include cancer patients and those receiving antibiotics and supraphysiologic doses of corticosteroids or other immunosuppressants.<sup>6</sup> Clinical and laboratory studies have shown that neutrophils play a major role in host defense against systemic candidiasis,<sup>8</sup> although recent work suggests that there is also a role for T cells and their cytokines in recovery from this disease.<sup>9</sup> Oral candidiasis, however, has been consistently associated with defects in the cell-mediated arm of the immune response.<sup>8</sup> In addition, candidal infections are believed to be more frequent in people with diabetes.<sup>10-12</sup> It has been suggested that the highest rate of colonization occurs in diabetic patients with poor serum glucose control,<sup>13,14</sup> although proof supporting this association is lacking.<sup>10,11</sup> The purpose of this study was to define the prevalence of candidal colonization in people with diabetes mellitus (DM) versus controls and to identify factors predisposing to colonization in the diabetic patient.

### Methods

Oral cytology was performed on 100 patients with type II DM on presentation to the diabetes center and 100 age and sex-matched non-diabetic controls. Mucosal scrapings were obtained from the buccal mucosa and posterior dorsum of the tongue of each patient and control subject with a tongue blade moistened with water. These scrapings were cultured on Sabouraud Dextrose Agar and then smeared over a glass microscope slide and sprayed with a commercially prepared fixative (KOH). All slides were examined microscopically for the presence of *Candida*. All evaluations were performed in a blind manner by one of the authors. The findings were grouped into the following categories; 0: negative, adequate numbers of epithelial cells with no evidence of fungi

on cytological examination and no growth on Sabouraud dextrose agar, 1: positive colonization, scattered collections of yeast forms in association with epithelial cells or variable numbers of intertwining pseudo-mycelial forms on cytological examination and growth of *Candida albicans* on Sabouraud Dextrose Agar. On interview, information was obtained from the study participants on the age, sex, and type and duration of diabetes. A detailed history was taken with regard to current and last month medications, with particular attention to the use of antibiotics or immunosuppressant therapy. Cardiovascular disease and hypertension were stated from medical history and when patients on regular chronic treatment for these conditions. An oral soft tissue examination was performed to rule out clinical candidiasis. A white blood cell count and differential, serum glucose (performed by glucose oxidase method), GHb value (HbA1c from ion-exchange chromatography), blood urea and serum creatinine were also obtained. A complete history and oral examination were obtained from the control group. Controls that were on immunosuppressant drugs or had a history of recent antibiotic use or malignancy or had pregnancy were excluded from the study. Data analysis was carried out by multiple comparisons using the statistical package for social sciences (version 18). Differences were considered as statistically significant at  $P \leq 0.05$ .

### Results

The mean age of the study population was 57 year, and the controls had a mean age of 51 year. In both the diabetic and control populations, the male-to-female ratio was 2:1. Differences in the distribution of *Candida* colonization between diabetic patients and controls were statistically significant ( $P < 0.005$ ) as shown in Table 1. Oral examinations revealed no clinical infections in either the study or control groups, indicating that the colonization was subclinical. In the study population the

primary reasons for consultation were poorly controlled diabetes mellitus (78%), acute infection (12%) and chronic infection (6%). Other common secondary problems on admission included hypertension, peripheral vascular disease, retinopathy and neuropathy. Evaluation of variables in Table 2 (including age of the patients, glycosylated Hb, blood sugar, duration of

diabetes, total white blood cells, total neutrophils, and total lymphocytes) were performed. All of these variables had no significant impact on the study. The mean level of blood urea of the studied patients was 35 mg/dl (range 15-84), while the mean level of serum creatinine of the studied patients was 0.95 mg/dl (0.3-2.8) as shown in Table 3.

**Table 1:** Comparison of negative and positive *Candida albicans* colonization in diabetic and control groups:

| <b>Candida albicans infection</b> | <b>Diabetic (100)</b> | <b>Control (100)</b> | <b>Total</b> |
|-----------------------------------|-----------------------|----------------------|--------------|
| <b>Negative</b>                   | 44 (44%)              | 70 (70%)             | 114 (57%)    |
| <b>Positive</b>                   | 56 (56%)              | 30 (30%)             | 86 (43%)     |
| <b>Total</b>                      | 100 (100%)            | 100 (100%)           | 100 (100%)   |
| <b>P-value</b>                    | 0.002                 |                      |              |

**Table 2:** Means  $\pm$  Standard division of factors associated with candidal colonization in diabetic subjects (100 cases)

| <b>Isolation of Candida albicans</b> | <b>Age</b>        | <b>Glycosylated Hemoglobin (%)</b> | <b>Blood glucose (mg/100 ml)</b> | <b>Duration of diabetes (yr)</b> | <b>Total white blood cells</b> | <b>Segmented leukocytes</b> | <b>Lymphocytes</b> |
|--------------------------------------|-------------------|------------------------------------|----------------------------------|----------------------------------|--------------------------------|-----------------------------|--------------------|
| <b>Positive</b>                      | 57.04 $\pm$ 10.6  | 6.88 $\pm$ 2.18                    | 230.5 $\pm$ 102.4                | 4.13 $\pm$ 1.99                  | 7.87 $\pm$ 3.58                | 62.79 $\pm$ 13.67           | 28.5 $\pm$ 11.7    |
| <b>Negative</b>                      | 57.32 $\pm$ 11.01 | 7.39 $\pm$ 2.50                    | 233.6 $\pm$ 90.5                 | 3.75 $\pm$ 1.34                  | 8.14 $\pm$ 2.63                | 65.7 $\pm$ 12.89            | 26.1 $\pm$ 11.8    |
| <b>P value</b>                       | 0.89              | 0.28                               | 0.72                             | 0.28                             | 0.67                           | 0.28                        | 0.32               |

**Table 3:** Renal function tests of the studied patients.

| <b>Candida albicans infection</b> | <b>Mean and range of blood urea (mg/dl)</b> | <b>Mean and range of serum creatinine (mg/dl)</b> |
|-----------------------------------|---|---|
| Positive                          | 38 (20-75)                                  | 1.08 (0.4-2.8)                                    |
| Negative                          | 32 (15-84)                                  | 0.84 (0.3-2.3)                                    |
| Total                             | 35 (15-84)                                  | 0.95 (0.3-2.8)                                    |

**Table 4:** Relations of some variables with *Candida albicans* colonization in diabetic group (100 cases)

| Candida albicans infection | Gender        |               | Smoking Habit |               | Receiving Antibiotic |               | Chronic renal disease |             | Cardiovascular disease |               | Hypertension  |               |
|----------------------------|---------------|---------------|---------------|---------------|----------------------|---------------|-----------------------|-------------|------------------------|---------------|---------------|---------------|
|                            | Male          | Female        | Yes           | No            | Yes                  | No            | Yes                   | No          | Yes                    | No            | Yes           | No            |
| <b>Negative</b>            | 10<br>(32.3%) | 34<br>(49.3%) | 6<br>(40%)    | 38<br>(44.7%) | 1<br>(25%)           | 43<br>(44.5%) | 5<br>(22.7%)          | 39<br>(50%) | 5<br>(35.7%)           | 39<br>(45.3%) | 24<br>(43.6%) | 20<br>(44.4%) |
| <b>Positive</b>            | 21<br>(67.7%) | 35<br>(50.7%) | 9<br>(60%)    | 47<br>(55.3%) | 3<br>(75%)           | 53<br>(55.2%) | 17<br>(77.3%)         | 39<br>(50%) | 9<br>(64.3%)           | 47<br>(54.7%) | 31<br>(56.4%) | 25<br>(55.6%) |
| <b>P. value</b>            | 0.08          |               | 0.48          |               | 0.40                 |               | 0.01                  |             | 0.35                   |               | 0.54          |               |

Isolation of *Candida albicans* was evaluated regarding to gender, recent antibiotic, smoking habit and chronic illness including chronic renal disease, cardiovascular disease and hypertension. No significant correlation was found between isolation of *Candida albicans* and these variables except for chronic renal disease which were significant (Table 4). No patient in the control group was under therapy.

### Discussion

Much has been written about candidiasis in the diabetic patients. Most statements made have been in reference to vulvovaginal infections. These observations are primarily supported by empirical clinical observations, and there has been a paucity of properly controlled experimental studies.<sup>10</sup> We thought that the oral mucosa of the DM patient is another likely site of candidal colonization and chose to investigate this further. We have used oral cytological smears as a simple, reproducible means of demonstrating a significant increase in frequency of candidal colonization in diabetic patients with confirmation by Sabouraud Dextrose Agar culture and compared the results with age and sex matched healthy controls. With well-defined microscopic criteria, the cytological smear technique can semiquantitatively illustrate actual candidal colonization. Therefore, the cytological approach is not only valuable for diagnosis but may play an equally

important role in defining more fundamental pathogenetic aspects of the infection.<sup>7</sup> Several parameters were evaluated to identify overt factors responsible for this increased colonization in diabetic patients. Although examination of total and differential white blood cell counts revealed no correlation to colonization status, the complex nature of the defense mechanisms against the *Candida* organism suggests that covert functional defects of neutrophils<sup>15-20</sup> or cell-mediated immunity<sup>21-24</sup> may play an important role in this population. Many patients with defects in neutrophil and macrophage function are susceptible to oral candidiasis.<sup>24</sup> The predisposition of the diabetic patient to infection by pathogenic fungal species has been explained in terms of enhancement of yeast growth by elevated tissue fluid glucose levels.<sup>25</sup> In addition, a good correlation between salivary glucose and *Candida* growth has been demonstrated in diabetic patients.<sup>26</sup> Thus, a correlation between diabetic control and extent of oral mucosal yeast colonization would be expected. We evaluated fasting serum glucose levels on admission because subsequently these levels were rapidly adjusted for optimal control. GHb levels were evaluated as an index of control over a more extended period of 2-3 months.<sup>27</sup> However, we were unable to correlate diabetic control with frequency of colonization, As other studies have shown.<sup>28</sup> The

availability of salivary glucose may influence *Candida* growth during antibiotic administration due to a selective reduction in oral micro flora and subsequent decrease in competition for the nutrient.<sup>25</sup> Diabetic patients receiving antibiotics might be expected to demonstrate increased candidal colonization.<sup>25</sup> We were unable to establish any correlation. Also we failed to correlate candidal colonization with the duration of diabetes.

### Conclusion

Despite solid evidence that candidal colonization is more prevalent in patients with DM than in age and sex matched controls, the factors responsible are largely unknown. Although fragmentary and circumstantial evidence of immunologic and metabolic defects has been found, a broader perspective on this infection remains elusive.

### Conflicts of interest

The authors report no conflicts of interest.

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