The Adhesion of Candida albicans to Epithelial Cells in Diabetes Mellitus Patients

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ABSTRACT

We determined the adhesion of Candida albicans to oral epithelial cells in diabetic patients by an autologous adhesion assay with exfoliated buccal or palatal epithelial cells and one strain of C. albicans isolated from each patient by concentrated oral rinse technique, and investigated the influence of the carbon source of the growth medium, source of epithelial cells, and the influence of gender and smoking on the adhesion. Glucose, sucrose and galactose were used as the predominant carbon source of the growth medium. The autologous strain of C. albicans adhered selectively to the oral mucosa of diabetic patients. Palatal epithelial cells retained significantly more C. albicans in vitro and adhesion influenced by the availability of sugars in the growth medium.

INTRODUCTION

Candida albicans is the most common fungal pathogens isolated from the oral cavity. One of the main causes of oral in candidasis is the presence of grant amount of carbohydrates in the oral cavity (1). The susceptibility of diabetic patients to cutaneous vaginal and oral candidosis has been well documented (2) and has been linked to the ability of C. albicans to adhere to mucous membranes. The importance observation that in the presence of 20 mM glucose the expression of iG3b receptor on C. albicans was doubled (3). This relevance in diabetic patients whose oral cavity is exposed to increased glucose levels in saliva (4). The capacity of yeast to disease depends on its ability to survive and thrive in special microenvironments within the host including the mucosa, and on a number of virulence factors that aid the pathogen's adherence to and invasion of host tissue and cells (5). This study investigated factors that may affect the adhesion of C. albicans to epithelial cells of insulin-using diabetic mellitus patients.
MATERIALS AND METHODS

The present study included 108 diabetic patients, none of subjects had received antibiotic, corticosteroid or antifungal therapy. *Candida albicans* was isolated from each patient by concentrated oral rinse technique (6). *Candida albicans* was identified by germ-tube formation (7) and by yeast identification system API 20C AVX (8).

Adhesion of the subjects own strain of *C.albicans* to their buccal and palatal epithelial cells was determined by an autologous adhesion assay. The adherence of *C.albicans* to epithelial cells was measured *in vitro* after growth the yeast to stationary phase in defined medium contains glucose, sucrose, and galactose as the carbon sources. An over night culture of *C. albicans* in sabouraud's dextrose was harvested and adjusted to $10^7$ *C. albicans* / ml, and 1ml was incubated with 1ml of suspension containing $10^5$ buccal and palatated cells obtained by gently rubbing the oral mucosa with sterile cotton swabs, followed by dispersion in sterile phosphate-buffered saline (PBS, pH 7.2) (9). The mixture was incubated in an orbital shaker operating at 150 rpm at 37° C for 1 hr. The epithelial cells with attached yeast were harvested and washed with 5 ml volumes of PBS on polycarbonate filter. The filters were air-dried fixed in methanol at room temperature, stained with Gram crystal violet and examined under a microscope. The number of *C.albican* cells attaching to 100 single epithelial cells was counted.

The effects of addition of sugars to the growth medium and source of epithelial cells were evaluated in the adhesion assay. The reproducibility of the autologous assay was determined as was the association between the adherence of *C.albicans* and patient parameters such as gender and smoking. Candidal colonization determined by counting the number of colonies on the primary isolation plate with colony counter and converted to CFU/ml of oral rinse.

RESULTS AND DISCUSSION

The study group was 108 diabetic patients (20-70) years old, mean (45) years, female 69(69%), male 39 (36.01%). *C.albicans* isolated from patients & identified by characters on sabourauds agar and identification system API 20C AUX (figure 1).
(+)=Positive.  (-)=Negative.

Figure -1:Identification of *C.albicans* by API 20C AUX.

The adherence of *C.albicans* to epithelial cells was measured *in vitro* after growth the yeast to stationary phase in medium contains glucose,sucrose,and galactose as the carbon source. The rate of yeast formation grown in medium contains 500 mM concentration of the different sugars correlated well with the relative adherence of the *C.albicans* to epithelial cells (Table 1) compares the adherence of *C.albicans* grown in medium contains 500 mM concentration of different sugars as the carbon source.

Table-1: Adherence of *C.albicans* grown in media containing different sugars as carbon source to oral epithelial cells:

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Mean (SD)/adhesion of yeast cells/100 epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>202 (124)*</td>
</tr>
<tr>
<td>Galactose</td>
<td>191 (101)</td>
</tr>
<tr>
<td>Glucose</td>
<td>104 (79)</td>
</tr>
</tbody>
</table>

*  Significant at the (P<0.05).

Adherence was enhanced according to the carbon source used. Sucrose & galactose were the most. The specific role of the organism to synthesize etherize extracellular glucans from this effective sugars of these tested. The specific role of the organism to synthesis extracellular glucans from this sugars which inturn allow it to accumulate on the tooth surface.

The palatal epithelial cells retained more *C.albicans*, but there was no demonstrable association between the adhesion of *C.albicans* to buccal epithelial cells and oral candidiasis, (table 2), gender & smoking were not associated with a significant increase in the adhesion of *C.albicans* to buccal epithelial cells of patients.
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Table -2: Adhesion of C.albicans to oral epithelial cells:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (SD) number of yeast cells/epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal epithelial cells</td>
<td>1.21 (2.11)</td>
</tr>
<tr>
<td>Palatal epithelial cells</td>
<td>8.85(12.72)*</td>
</tr>
</tbody>
</table>

* Significant at the (P<0.05).

The current study reveled increase in the adhesion of C. albicans to buccal epithelial cells when sugars (sucrose, galactose) were added to the growths medium. Galactose was the most effective sugar of these tested, yeasts harvested from medium containing 500mM galactose showed more than 10 fold greater adherence then cells grown in medium with 50mM glucose (1,19). Mc Courtic and Douglas (12) reported a five-fold increase in adhesion when isolates C. albicans from patients with active infection were grown in broth containing 500mM sucroce. The effect of sugars can be due to the production of a mannoprotein surface layer which is known to enhance adhesion (B), which is virtually absent in yeast grown in media containing 50mM glucose (14). It is possible that the accumulation of glycosylation products in epithelial cells may increase the number of receptors for C. albicans on epithelial cells surfaces. Also high salivary glucose levels which are commonly seen in diabetic patients (3,15) may produce increased resistance to intracellular killing by phagocytosis (16).

We used autologus adhesion assay system (i.e. both buccal cells and C. albicans isolated from the same patients). Epithelial cells were used because they represent a natural mucocutaneous surface which is a common site for candidal infection in vivo. A condida albicans isolate from each patient was used as the test organism and also has been shown to adhere to buccal epithelial cells (8, 17).

This agreement with (15) that the present study observated that in vivo palatal cells retained significantly more C. albicans than buccal cells. C. albicans is most prevalent in the palate, tongue & gingival. The palatal cells which are more resistant to the stringent washing procedures used in autologoul adhesion assy system, and the palate is a common site for intraoral candidal infection.

The results of the present study could also been showed that the mean condidal adhesion to buccal epithelial cells of females was higher than in males but the difference between the groups was not significant. This at has also been reported for healthy individuals (18). Smoking increased the number of C. albicans cells attaching to buccal epithelial cells of diabetic patients. It has been suggested that tobacco smoking may lead to localized epithelial alterations that facilitate Candida adherence (19).
REFERENCES


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