Factors affecting the adhesion of *Candida albicans* to epithelial cells of insulin-using diabetes mellitus patients

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This study investigated the influence of the carbon source of the growth medium, strains of *Candida albicans* and source of epithelial cells, and the influence of smoking and gender, on the adhesion of *C. albicans* to epithelial cells from insulin-using diabetic patients. Adhesion was determined by an autologous adhesion assay with exfoliated buccal or palatal epithelial cells and one strain of *C. albicans* isolated from each patient. The type strain CBS 562 was also used. Glucose or sucrose were used as the predominant carbon sources 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 vivo and adhesion was influenced by the availability of sugars in the growth medium and the strain of *C. albicans*.

Introduction

The susceptibility of diabetic patients to cutaneous, vaginal and oral candidosis has been well documented [1–3] and has been linked to the ability of *Candida albicans* to adhere to mucous membranes. Of particular importance is the observation that in the presence of 20 mM glucose, the expression of the iC3b receptor on *C. albicans* was doubled [4]. This may have particular relevance in diabetic patients, whose oral cavity is exposed to increased glucose levels in saliva [5].

With the increased awareness of candidosis as a clinical disease, several studies have documented the adhesion of *C. albicans* to various epithelial surfaces. These have included epithelial cells [6–9], human vaginal cells [10], uro-epithelial cells [11] and epithelial cell lines [12, 13]. Such studies have shown that factors associated with the yeast cells, epithelial cells and the environment all affect yeast attachment in vivo. This study investigated factors that may affect the adhesion of *C. albicans* to epithelial cells of insulin-using diabetes mellitus patients.

Materials and methods

Subjects of the study

A total of 120 diabetic patients was recruited from outpatient Diabetic Clinics at Belfast City Hospital and the Royal Victoria Hospital, Belfast, while attending for routine 3-monthly review. None of the subjects had received antibiotic, corticosteroid, or antifungal therapy during the preceding 3 months. All subjects gave informed consent to participate in the study, which was approved by the local Research Ethics Committee. *C. albicans* was isolated from each patient by a concentrated oral rinse technique [14].

Adhesion of the subjects own strain of *C. albicans* to their buccal and palatal epithelial cells was determined by an autologous adhesion assay. An overnight culture of *C. albicans* in Yeast-bacteriological Peptone-Dextrose (YPD) broth (Oxoid) or Sabouraud’s dextrose broth was harvested and adjusted to $10^7$ *C. albicans*/ml (determined by microscopy) and 1 ml was incubated with 1 ml of a suspension containing $10^5$ buccal or palatal cells, obtained by swabbing the buccal and palatal mucosa and passing through a 23 gauge needle. The incubation fluid was phosphate-buffered saline (PBS; 0.2 M, pH 7.2). The mixture was incubated in an orbital shaker operating at 80 rpm at 37°C for 1 h (Gallenkamp, Loughborough). The epithelial cells with attached yeast were harvested and washed six times with 5-ml volumes of PBS on a 12-μm (25 mm diameter) polycarbonate filter (Nucleopore GMBh,
Germany) by negative pressure from a Venturi pump. The filters were air-dried, fixed in methanol at room temperature, stained with periodic acid/Schiff reagent (BDH) and counter-stained with haematoxylin (BDH). The number of C. albicans cells attaching to 100 single, complete epithelial cells was counted.

The reproducibility of the autologous assay was determined, as was the association between the adherence of C. albicans and patient characteristics such as smoking, gender, the magnitude of candidal colonisation - determined by counting the number of colonies on the primary isolation plate with a Protus colony counter (Synoptics, Cambridge) and converted to cfu/ml of oral rinse [14] - and clinical presentation. The effects of addition of sugars to the growth medium and the source of epithelial cells were also evaluated in the adhesion assay and the results obtained with the patients' strains were compared with adhesion of the type strain of C. albicans (CBS 562).

Statistical analysis

Reproducibility studies were analysed by coefficient of variation. Factors affecting adhesion were determined statistically by analysis of variation (ANOVA) followed by Scheffe's F test and the paired and unpaired Student's t test. Probabilities of <5% were taken to be statistically significant.

Results

A total of 120 diabetic patients participated in the study. The mean age was 49 SD 15 years; 73 patients were male and 47 were female. The coefficient of variation (0.8) was identical when the adhesion assay was repeated independently on two occasions for 21 variation (0.8) was identical when the adhesion assay was repeated independently on two occasions for 21 variation (0.8) was identical when the adhesion assay was repeated independently on two occasions for 21 years; 73 patients were male and 47 were female. The coefficient of variation (0.8) was identical when the adhesion assay was repeated independently on two occasions for 21 samples, illustrating the reproducibility of this method.

No significant difference in adhesion of C. albicans to buccal epithelial cells of diabetic patients was observed when YPD broth or Sabouraud's dextrose broth (which both contain glucose as the predominant carbon source) was used. However, when C. albicans was grown in Sabouraud's broth containing 500 mM sucrose, the mean adhesion was 210 SD 131 yeast cells/100 buccal epithelial cells, compared with 127 SD 101 yeast cells/100 buccal epithelial cells for YPD broth and 102 SD 80 yeast cells/100 buccal epithelial cells for Sabourauds' dextrose broth. This difference was significant (p < 0.05).

Each patient's own strain of C. albicans adhered in significantly greater numbers than C. albicans CBS 562. 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 candidosis (Table 1). Smoking, gender and magnitude of candidal colonisation were not associated with a significant increase in the adhesion of C. albicans to buccal epithelial cells of diabetic patients.

Discussion

A reproducible autologous adhesion assay system (i.e., both buccal cells and C. albicans isolates were from the same subject) was used in this study of C. albicans adhesion in diabetic patients. Epithelial cells were used because they represent a natural mucocutaneous surface, which is a common site for candidal infection in vivo. Large numbers of cells can be collected by swabbing the mucosa, and the size and morphology of the cells, coupled with the observation that there are few indigenously attached bacteria, make them suitable for the counting procedures. A C. albicans isolate from each patient was used as the test organism, as not only is this the candidal species isolated most frequently in episodes of oral candidosis [15], it also has been shown to adhere to buccal epithelial cells in greater numbers than other candidal species [10].

In the present study there was a two-fold increase in the adhesion of C. albicans to buccal epithelial cells when sucrose was added to the growth medium. McCourtie and Douglas [16] reported a five-fold increase in adhesion when isolates of C. albicans from patients with active infection were grown in broth containing 500 mM sucrose. This increase in adhesion has been attributed to the production of a fibroflocular layer on the yeast cell surface containing carbohydrate, protein, phosphorus and glucosamine [17], which is virtually absent in yeast grown in media containing 50 mM glucose [18]. It is possible that the accumulation of glycosylation products in epithelial cells may increase the number of receptors for C. albicans on epithelial cell surfaces. Also, high salivary glucose levels, which are commonly seen in diabetic patients

<table>
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<tr>
<th>Parameter studied</th>
<th>Mean (SD) number of C. albicans/epithelial cell</th>
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<tr>
<td>Autologous versus type strain of C. albicans</td>
<td>70.42 (55.97)* versus 28.13 (29.83)</td>
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<tr>
<td>Buccal versus palatal epithelial cells</td>
<td>1.43 (2.21) versus 10.58 (14.94)*</td>
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<tr>
<td>Healthy oral mucosa versus oral candidosis</td>
<td>107 (58.17) versus 127.7 (94.75)</td>
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*Significant at the 5% level.
Adhesion may also be affected by the candidal strain. Traditionally, adhesion assays have used a reference strain of C. albicans such as CBS 562, which was originally isolated from a patient with cutaneous candidosis. In the present study, the subject’s own strain of C. albicans bound in significantly higher numbers than the type strain. This suggests that within the host there is strain specificity of adherence of C. albicans to host buccal epithelial cells. This may be attributed to an ability of the host C. albicans strain to modify its surface composition in response to high salivary glucose concentrations in the oral cavity of diabetic patients. The type strain may lack this capability, or possess it to a lower degree. Therefore, the autologous assay is likely to provide a more accurate representation of the interaction of C. albicans and epithelial cells in the host.

Although the mean candidal adhesion to buccal epithelial cells of females was higher than the corresponding values found in males, the difference between the groups was not significant. This has also been reported for healthy individuals [19]. No significant relationship was observed between the wearing of a denture and candidal adhesion to buccal epithelial cells of diabetic patients. 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 localised epithelial alterations that facilitate candidal adherence [20]. No significant difference was observed in the ability of C. albicans isolates from diabetic patients with a healthy oral mucosa, or C. albicans isolates from patients with oral candidosis to bind to host buccal epithelial cells. This is in agreement with the findings of Darwazeh et al. [8] and suggests that oral candidal infection cannot be explained in terms of an increase in the receptivity of buccal epithelial cells for C. albicans.

In conclusion, the present study has demonstrated a simple and reproducible method for investigating candidal adhesion in vitro. In patients with insulin-requiring diabetes mellitus, the patient’s own strain of C. albicans adhered in significantly greater numbers than the reference strain. Palatal epithelial cells retained C. albicans in greater numbers in vivo, compared with buccal epithelial cells. Adhesion was also shown to be significantly affected by the carbon source of the growth medium and by smoking. This suggests that the increased incidence of candidal carriage and oral candidosis in diabetic patients is multifactorial and may be associated specifically with an increased sugar content in saliva.

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References