

Comparison of *Candida albicans* Adherence to Human Corneocytes from Various Populations

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Epidemiological data indicate that patients suffering from diabetes, hypothyroidism, obesity, or following prolonged treatment with antibiotics, corticosteroids or oral contraceptives, have an increased tendency to develop cutaneous candidiasis.

Since it is generally believed that attachment of microorganisms to host cells is an initial step in the evolution of infection, the aim of the present study was to investigate whether cells from susceptible individuals have increased capacity to bind the fungus. Corneocytes collected from the forearms of individuals of these susceptible groups were exposed in vitro to *Candida albicans* and adhesion to the cells was evaluated in comparison with adherence to cells from a non-susceptible population. Adherence in vitro was assayed microscopically and evaluated quantitatively by two parameters: 1) percentage of adherence - number of corneocytes with adhering yeasts on their surface, and 2) the total number of adhered yeasts. Results of the study revealed that the mean percentage of adherence and the mean total number of yeasts adhering to cells from individuals of the susceptible populations was twice as high as values in a healthy population. Statistical analysis of the data by Student's *t*-test indicated that the difference was significant ($p < 0.001$). **Key words:** Fungus; Attachment; Stratum corneum cells.

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Superficial candidiasis is an opportunistic infection caused by the commensal *Candida* species, which can colonize human oral, gastrointestinal, vaginal and cutaneous epithelium (1, 2). Most *Candida* infections, including cutaneous candidiasis, are caused by *C. albicans*, although other *Candida* species have also been incriminated.

There are numerous conditions that predispose individuals to *Candida* infection, including diabetes, obesity, use of oral contraceptives, broad-spectrum

antibiotics, prolonged systemic steroid therapy, or endocrinologic disturbances such as hypothyroidism, hypoparathyroidism and hypogonadism (1, 2).

It is generally accepted that the infectious process is initiated by the attachment of the microorganism to the host's epithelial surfaces, and is followed by colonization, and eventual invasion. The attachment protects the microbial parasite from elimination by host defence mechanisms (3).

In view of the increased incidence of infections in certain populations, as mentioned above, we initiated a study to investigate whether corneocytes from individuals of these groups have a higher capacity to bind the fungus. We compared in vitro adherence of *C. albicans* to corneocytes of populations prone to cutaneous candidiasis with that of cells from an unsusceptible population. The results of this investigation enable us to give some explanation for the phenomenon of increased incidence of cutaneous candidal infections in certain populations.

MATERIAL AND METHODS

Patients

Corneocytes were collected from outpatients of the Department of Dermatology at Ichilov Hospital, Tel-Aviv Sourasky Medical Center, suffering from diabetes, hypothyroidism or obesity, or those taking broad-spectrum antibiotics, systemic steroids and women using oral contraceptives for more than a month. Healthy volunteers of the Tel-Aviv Sourasky Medical Center staff served as controls.

Corneocytes

Corneocytes were obtained by a method based on the technique of Collins-Lech et al. (4). Briefly, the cells were collected by gentle scraping of the proximal forearm with a Teflon policeman into 2 ml of phosphate-buffered saline (PBS) containing 0.1% Triton X-100. Following three washings with PBS, the cells were resuspended to a concentration of 1×10^6 cells/ml.

Yeasts

C. albicans CBS-562, from The Centraal Bureau voor Schimmel Cultures in Delft, Holland, was used throughout this study. The culture was maintained at 4°C and subcultured at 28°C on Sabouraud's dextrose agar, containing

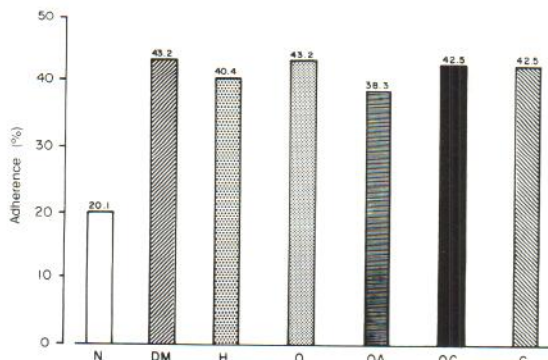


Fig. 1. In vitro adherence of *C. albicans* to corneocytes. Percentage of adherence. Statistical analysis, $p < 0.001$ (Student's *t*-test). N, normal; DM, diabetes mellitus; H, hypothyroidism; O, obesity; OA, oral antibiotics; OC, oral corticosteroids; C, contraceptive pills.

chloramphenicol. For adherence assays, the fungus was grown as in previous studies (5, 6), in a liquid yeast extract medium at 28°C for 18 h under constant shaking. The cultures were harvested by centrifugation, the blastospores were washed three times in PBS and resuspended to a concentration of 1×10^8 organisms/ml.

Adherence assay

The adherence assay used constitutes a modified combination of the technique of Collins-Lech et al. (4), and of a method used by us for studying yeast adherence to vaginal cells (5, 6). Specifically, samples of 0.25 ml of *C. albicans* and 0.25 ml of corneocytes, prepared as described above, were mixed in siliconized tubes and incubated on a rotator at 37°C for 2 h. Wet mounts were prepared from the adherence mixture immediately following incubation, by placing samples of 50 µl on cleaned microscopic slides covered with coverslips.

Adherence was evaluated microscopically by two criteria: percentage of adhering corneocytes and total number of adhered yeasts. In order to avoid counts of corneocytes with accidental contact with yeasts, only corneocytes with six or more organisms on their surfaces were considered as adhering cells. This evaluation-method was compared with evaluation following filtration on the adherence mixture through 12-µm pore-size filters, and no significant differences between the two techniques were found. Therefore, the method of direct sampling was employed throughout the study. Percentage of adherence was established by counting the number of adhering corneocytes per 100 cells. Total number of adhered yeasts was calculated by counting the number of yeasts which attached themselves to the adhering corneocytes. Each tube was read in duplicate.

Evaluation of data

For statistical analysis of the data, Student's *t*-test was used for comparison of each test group with the control. Probability levels of $p < 0.05$ were used as indication for statistical significance.

RESULTS

Adherence of *C. albicans* to corneocytes from diabetic patients

The ability of corneocytes taken from 29 diabetic patients (25 to 85 years old; 17 females, 12 males) to adhere *C. albicans* was examined. Diabetes mellitus was established by the criteria of the WHO and Diabetes Data Group of NIH: 140 mg/dl venous plasma glucose following overnight fasting and 200 mg/dl after 2 h post-ingestion of 75 g glucose (7).

The ability of corneocytes from each patient to adhere to the fungus was measured by the two parameters described in Methods: the percentage of adherence of corneocytes, and the total number of adhered yeasts. The experiment was conducted in duplicate or triplicate. As shown in Figs. 1 and 2, the mean adherence percentage of the 29 patients was 43.2% (SD = 14.9) and the mean total number of the adhered yeasts was 394.9 (SD = 196.3), while the corresponding values of the controls were 20.1% (SD = 8.0) and 153.3 cells (SD = 77.1), respectively. The difference between the values of the diabetic patients and those of the healthy volunteers was significant ($p < 0.001$).

Adherence of *C. albicans* to corneocytes from hypothyroid patients

Corneocytes taken from 15 female patients (age: 25–80 years) with hypothyroidism, were tested for their ability to adhere *C. albicans*. Criteria for hypothyroidism were established by serum analysis of T3, T4, TSH. The mean percentage of adherence was 40.4 (SD = 16.5) and the total number of the adhered yeasts was 338.2 (SD = 181.1). (Figs. 1 and

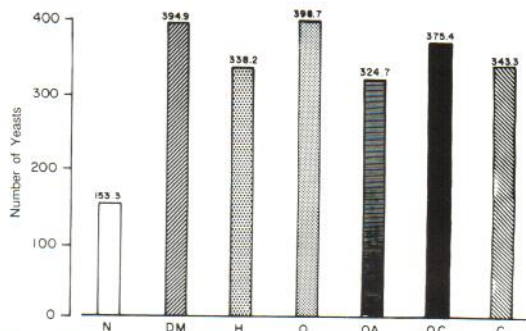


Fig. 2. In vitro adherence of *C. albicans* to corneocytes. Number of adhered yeasts. Statistical analysis, $p < 0.001$ (Student's *t*-test). N, normal; DM, diabetes mellitus; H, hypothyroidism; O, obesity; OA, oral antibiotics; OC, oral corticosteroids; C, contraceptive pills.

2). The difference between the values of the hypothyroid patients and those of the controls was statistically significant ($p < 0.001$).

Adherence of C. albicans to corneocytes from obese patients

Corneocytes from 17 obese patients (8 males and 9 females, age range 36 to 79 years) were tested. Obesity was determined by superabundance in adipose tissue, expressed in a preponderance of at least 20% of the ideal weight of the examined (8). The values of the two parameters used to evaluate the ability of the corneocytes to bind the fungus, were 43% (SD = 15) adherence, and 398 (SD = 176.8) attached organisms, respectively (Figs. 1 and 2).

Adherence of C. albicans to corneocytes from patients treated with antibiotics

Corneocytes from 14 patients taking systemic antibiotics (for more than a month) due to: infected leg ulcer (2 patients), pneumonia (2 patients), deep vein thrombosis (2 patients), erysipelas (2 patients), chronic urinary tract infection (1 patient) and acne (5 patients), were tested. Seven patients out of these 14 were treated with penicillin or its derivatives, 6 with tetracycline or its derivatives, and one patient received sulphamethoxazole.

The mean adherence percentages and the mean total number of yeasts adhering to corneocytes of patients on systemic antibiotics were 38.9% (SD = 13.8) and 324.7 (SD = 122.7), respectively, again demonstrating a significant difference ($p < 0.002$) in comparison with the control.

Adherence of C. albicans to corneocytes from patients treated with corticosteroids

Corneocytes from 15 patients (8 males and 7 females) on oral corticosteroids for at least a month, due to pemphigus vulgaris (2 patients), rheumatoid arthritis (8 patients), Crohn's disease (2 patients), or bronchial asthma (3 patients), were examined. The adherence ability of the corneocytes from these patients was 42.5% (SD = 14.5) and adhered yeasts 375.4 (SD = 157.5) (Figs. 1 and 2).

Adherence of C. albicans to corneocytes from women using oral contraceptives

We examined the in vitro adherence of *C. albicans* to corneocytes collected from 15 women from the 16–30 year age group, using oral contraceptives for more than a month: Diane (cyproterone acetate and

ethinyl-oestradiol), Logynon (levonorgestral and ethinyl-oestradiol), Microgynon (levonorgestral and ethinyl oestradiol), or Neogynon (*d*-norgestrol and ethinyl oestradiol). The mean adherence values of this group were: 42.5% adhering corneocytes (SD = 12.4) and 343.3 adhered yeasts (SD = 145.3). Here too, the statistical analysis of the data revealed a significant difference ($p < 0.001$) versus the control.

Adherence of C. albicans to corneocytes from normal controls

The control group included 31 healthy volunteers from the Tel-Aviv Sourasky Medical Center staff (14 males and 17 females). None of the female volunteers was pregnant or used oral contraceptives. A mean value of 20.1% corneocytes (SD = 8.0) from these individuals revealed the ability to adhere at least 6 *C. albicans* yeasts and a mean of 153.3 (SD = 77.1) yeasts adhered to these corneocytes.

DISCUSSION

The ability of microorganisms to colonize epithelial surfaces is associated with the capacity to attach to these surfaces (9). The correlative relation between in vitro adhesion and clinical conditions was reported in several studies. For instance: *Escherichia coli* cultivated from patients with acute pyelonephritis adhered more to epithelial cells of the urinary tract than *E. coli* cultivated from asymptomatic patients (10). A correlation was also observed between the ability of the host's epithelial cells to bind microorganisms and the ability of the host to develop certain diseases. For instance, urinary tract epithelial cells from women with recurrent urinary tract infections revealed higher levels of adherence of *E. coli* than cells from asymptomatic women (11).

We have shown in a previous study (6), that vaginal epithelial cells collected from women known to have increased predisposition to vaginal candidiasis, such as diabetics, pregnant women, or those using oral contraceptives, had an increased capacity to attach *C. albicans*. In the present study, we observed a similar correlative relationship, by demonstrating that cells from populations susceptible to cutaneous candidiasis such as diabetic, obese and hypothyroid patients, and individuals treated with antibiotics, steroids or oral contraceptives, have a two-fold higher ability to bind the fungus, than those from a healthy control population. The highest adherence values were found among the diabetics, and

in the obese population. Statistical evaluation of the data revealed that the differences between all the groups tested and the control group are significant.

Although *C. albicans* adherence to corneocytes, as cells of the orthokeratotic epidermis, may be expected to be different from cells of the parakeratotic epithelium, a clear-cut statistical correlation between epithelial cells adherence and epidermal corneocytes adherence was observed by Ray et al. (9). Thus, apparently the adherence mechanism is similar for these two cell types, despite the difference in their nature.

It is possible that specific physiologic and hormonal conditions such as diabetes, hypothyroidism, obesity, or use of oral contraceptives or steroids, may be expressed in an increased number or enhanced availability of fungal-binding sites on the epithelial host cells, thereby leading to increased adherence. However, it is difficult to explain why treatment with antibiotics should result in the increased adherence in an in vitro system, whereas its effects in vivo may be more plausible, taking into consideration various factors such as changes in normal flora.

It may be concluded that cells from susceptible individuals express a greater capacity to bind the fungal parasites, apparently leading to increased colonization and development of infection. These observations contribute to our understanding of the evolution of cutaneous candidiasis in susceptible individuals and in the future may eventually lead to attempts to inhibit adhesion and thereby prevent infection.

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