Quantitative Relationships Between *Candida albicans* in Saliva and the Clinical Status of Human Subjects

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Patients with candidiasis had >400 colony-forming units per ml of saliva, whereas carriers of *Candida albicans* had <400 colony-forming units per ml. Thus, quantitative cultures of saliva may aid in the diagnosis of oral candidiasis.

Of normal human populations, about 20 to 60% harbor *Candida albicans* intraorally without signs or symptoms of candidiasis (1-4, 6, 7, 9); therefore, positive nonquantitative cultures are of little value in diagnosing oral candidiasis. However, in our studies on the adherence of *C. albicans* to human buccal epithelial cells and the effects of salivary antibodies on adherence, we found a correlation between signs and symptoms of candidiasis and colony counts greater than 400 colony-forming units (CFU) per ml of saliva. Subjects without evidence of the disease, but who were classified as carriers because *C. albicans* grew from their saliva, had colony counts of less than 400 CFU/ml of saliva.

A total of 67 subjects with oral problems were seen by one investigator in the Oral Diagnosis Clinic at the University of Washington, School of Dentistry, Seattle. Mean age of the subjects was 52.1 years. A verbal health history and drug history was collected. Subjects reporting a history of antibiotic or drug use or medical conditions known to predispose to oral candidiasis (e.g., diabetes, oral contraceptives, steroid therapy, antibiotic therapy, etc.) were excluded from the study. A diagnosis of chronic or acute candidiasis was made on the basis of signs and symptoms and a positive culture, as shown in Table 1.

Saliva samples were collected under standard conditions, immediately placed on ice, and cultured within 1 h. Subjects not stimulated to salivate were asked to expectorate into sterilized, wide-mouthed centrifuge tubes all saliva during a 15-min period. Such unstimulated whole saliva is made up of secretions from the parotid, submandibular, and minor salivary glands. The 15-min period was used to estimate the flow rate of unstimulated saliva and to provide adequate volumes for the study.

A 0.5-ml amount of uncentrifuged saliva was spread on Sabouraud glucose agar plates containing 50 mg of chloramphenicol per ml; plates were incubated at 37°C for 48 h, and the number of CFU per milliliter of saliva was counted. Plates without fungal growth at 48 h were incubated for up to 2 weeks before being discarded as negative. The medium and culture conditions employed selected for yeast growth, and usually all the colonies were similar in appearance; therefore, one or a few colonies were subcultured and determined to be either *C. albicans* or not *C. albicans*. Yeasts were identified as *C. albicans* on the basis of positive germ tube formation, growth on cornmeal agar, and carbohydrate assimilation tests (8). Yeasts other than *C. albicans* were not further identified, and subjects with these yeasts were not included in this study. It is entirely possible that some of the subjects in the study could have been infected with other yeasts in addition to *C. albicans*, but all were shown to have *C. albicans* infections.

Figure 1 shows the mean numbers of CFU of *C. albicans* per milliliter of saliva from subjects classified on the basis of clinical signs and symptoms and a positive culture into three categories: group 2, carriers of *C. albicans*; group 3, patients with chronic candidiasis; and group 4, patients with acute candidiasis. The control group of subjects (group 1) had negative cultures for *C. albicans*. The variation was large in each group. Carriers had a mean of 244 CFU/ml (95% range, 111 to 378 CFU/ml), and values ranged from 2 to 888 CFU/ml of saliva. Patients with chronic candidiasis had a mean of 1,508 CFU/ml (95% range, 781 to 2,235 CFU/ml), with a range of 22 to more than 5,000 CFU/ml. Patients with acute candidiasis had mean colony counts of 3,549 (95% range, 1,375 to 5,724 CFU/ml) with a range of 156 to 9,824 CFU/ml.

Statistical analysis showed significant differences between colony counts in saliva from carriers and saliva from patients with either chronic candidiasis (P < 0.002) or acute candidiasis (P < 0.007), indicating that carriers and patients

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Table 1. Criteria for human subject classification

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Presence in subject group (no. of subjects)*</th>
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<tbody>
<tr>
<td></td>
<td>Control (21)</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>-</td>
</tr>
<tr>
<td>Symptoms</td>
<td>-</td>
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<td>Culture positive for C. albicans</td>
<td>-</td>
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<tr>
<td>Changes in signs or symptoms with treatment</td>
<td>-</td>
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</table>

*+ Criterion present; -, criterion absent; ±, criterion present or absent.

Fig. 1. Mean numbers (●) and 95% confidence intervals (bars) of CFU per milliliter of saliva from subjects of the four groups: (1) control; (2) carriers of C. albicans; (3) patients with chronic candidiasis; and (4) patients with acute candidiasis. All counts were done before treatment was started.

with disease can be distinguished within 95% confidence limits on the basis of quantitative cultures for C. albicans. The number 400 was taken as an arbitrary cut-off point between the two groups as statistical analysis indicated that individuals with <400 CFU/ml were classified as carriers and that those with >400 CFU/ml were classified clinically as having either chronic or acute candidiasis (P < 0.0001).

Thus, a positive correlation existed between large numbers (more than 400) of CFU of C. albicans per ml of saliva and the presence of signs and symptoms of candidiasis. Saliva from carriers of C. albicans without evidence of disease generally had less than 400 CFU of C. albicans per ml. Therefore, quantitative cultures of saliva represent a practical aid in the diagnosis of oral candidiasis when considered as part of an overall assessment of each individual.

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Literature Cited