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Recovery of *Candida dubliniensis* and Other Yeasts from Human Immunodeficiency Virus-Associated Periodontal Lesions

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Oral and subgingival samples from periodontal lesions were collected from 54 human immunodeficiency virus (HIV)-positive and 20 HIV-negative patients and cultured for yeast species. Of the 54 samples cultured from HIV-positive patients, 44 (82%) were positive for yeast species, of which 29 (66%) were subgingival. A total of 19 (48%) patients were positive for *Candida dubliniensis*, of which 15 (79%) were colonized in subgingival sites. Seven isolates of *Candida glabrata*, two isolates of *Candida parapsilosis*, and one isolate of *Saccharomyces cerevisiae* were recovered. This study reports for the first time the recovery of *C. dubliniensis* from subgingival intraoral sites and confirms the presence of *Candida* species in sites of periodontal disease associated with HIV.

Fungal species have gained considerable importance as opportunistic pathogens of immunocompromised individuals, particularly those infected with human immunodeficiency virus (HIV) (12, 28). Although *Candida albicans* is the most common etiologic agent of oral candidosis, *Candida dubliniensis* has emerged as another pathogen, noted for its in vitro potential for azole resistance and its enhanced in vitro adherence to human buccal epithelial cells (9, 14, 16, 17, 20, 23, 30).

The human oral cavity offers a unique environment with a multitude of ecological niches for microbial colonization. Periodontal diseases have gained attention in HIV-positive individuals as a result of the rapidly progressive and often destructive nature of the diseases (10). Periodontal diseases are characterized by an inflammatory, degenerative, and necrotic response in the gingival and underlying connective tissues, elicited by microbial colonization in periodontal pockets (3, 13). The initiation and progression of periodontal diseases are the result of the colonization and multiplication of microorganisms in subgingival sites, stimulation of the host immune system, and the release of host immune factors causing tissue damage (10). HIV-positive individuals and AIDS patients in particular often suffer from somewhat unique forms of periodontal diseases, the result of altered immune response in the periodontal tissues with changes in cellular and humoral immune responses (1, 2, 24, 27).

The cultivable microflora from HIV-positive patients with periodontal diseases has been found to be comprised of periodontopathic species similar to those found in HIV-negative subjects with periodontitis, mainly gram-negative bacteria (5, 24). Although the presence of yeasts was a striking feature of the subgingival plaque samples from some AIDS patients, follow-up studies confirming these preliminary data have been lacking (11, 27, 28, 32). The present investigation sought to begin to determine any association between the recovery of yeasts and the presence of advanced periodontal lesions. The study was designed to recover and identify yeast isolates from the oral tissues and subgingival sites of HIV-positive patients with advanced periodontal disease and to compare the results to those obtained from a group of 20 HIV-negative subjects with comparable clinical periodontal diseases.

Fifty-four adult patients with positive histories of HIV infection and 20 healthy nonimmunocompromised control subjects were recruited, and informed consent was obtained. Each patient had clinical evidence of at least one deep periodontal lesion (pocket depth, >5 mm). Periodontal evaluations of all patients included clinical examination and radiographs. The subjects ranged in age from 17 to 58 years (24% female and 76% male). At the time of collection, 14 patients had evidence of clinical mucosal oral lesions that were suggestive of candidosis. The HIV-negative control subject group was comparable with regard to age and sex, without clinical signs of fungal infection. With a sterile curette, subgingival samples were collected from periodontal sites (pocket depth, >5 mm), identified by measurement with a Marquis probe and by periodontal attachment loss as ascertained by clinical and/or radiological examination. In addition to subgingival samples, tongue or buccal mucosal (TBM) samples were obtained with a sterile swab. Subgingival samples were immediately streaked onto Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) for isolation; similarly, oral swabs were rolled on Sabouraud dextrose agar and streaked for isolation. Samples were collected from all patients by the same procedures. Agar plates were incubated at 35 to 37°C, in a non-CO_2 atmosphere, for 48 to 72 h and checked daily for growth. Fungal growth was subjectively rated as ranging from light to heavy.

All isolates growing on primary culture were identified by conventional mycology methods. Briefly, isolates that were germ tube positive were tested for their ability to grow at 45°C (*C. albicans* grows at 45°C, whereas *C. dubliniensis* fails to grow

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TABLE 1. Yeast species recovered from HIV-positive periodontal patients

Yeast species	Total no. of samples that were positive for yeast species by[a]:		
	TBM culture	Subgingival culture	TBM and subgingival culture
	44 (82)	29 (66)	29 (66)
C. albicans	26 (59)	16 (55)	13 (30)
C. dubliniensis	15 (34)	14 (48)	9 (31)
C. glabrata	6 (14)	5 (17)	4 (14)
S. cerevisiae and *C. parapsilosis*	1 (2)	0	0
C. parapsilosis	2 (5)	0	0
C. dubliniensis and *C. glabrata*	1 (2)	1 (3)	0
C. dubliniensis and *C. albicans*	1 (2)	2 (7)	1 (4)

[a] Values in parentheses are percentages.

at this temperature). Germ tube-positive isolates that failed to grow at 45°C were tested with the coaggregation assay, as described previously (15). CHROMagar Candida (CHROMagar, Paris, France) was used to identify yeast species that yielded negative results in germ tube culture and *C. dubliniensis* based on colony color. All isolates were tested for substrate assimilation profiles by the API 20C Aux system (bioMérieux Vitek, Inc., Hazelwood, Mo.) to identify germ tube-negative isolates to the species level and to confirm the identification of *C. dubliniensis*. All germ tube culture-positive isolates that failed to grow at 45°C were positive with the coaggregation assay and produced a dark green colony color in testing with CHROMagar Candida; these results were consistent with *C. dubliniensis* identification. The API 20C system confirmed the identification of *C. dubliniensis* and identified to the species level the germ tube-negative isolates.

The results of fungal cultures from the HIV-positive patients are summarized in Table 1. Among the 54 patient samples, a total of 44 (82%) were positive for yeasts; 29 (66%) of these were subgingival samples. *C. albicans*, *C. dubliniensis*, *Candida glabrata*, *Candida parapsilosis*, and *Saccharomyces cerevisiae* were the yeast species recovered. Two or three species were coisolated from eight patients, and subgingival and TBM samples of nine patients indicated colonization by different species. A total of 21 (48%) patients were found to be harboring *C. dubliniensis*; 15 (79%) samples from these patients were recovered from periodontal lesions, with seven exclusively in the subgingiva (Table 1). The 20 HIV-negative patients yielded six positive TBM samples and one positive subgingival sample, with light growth of *C. albicans. C. dubliniensis* was not recovered from the HIV-negative patients.

The oral cavity is a prime site for a variety of tissue lesions associated with HIV infection, including mucocutaneous and esophageal candidosis, sometimes as the first clinical expression of HIV infection (4, 18, 22, 25, 31, 32). Many studies have reported subgingival *C. albicans* in adult periodontitis, yet it is not known whether subgingival fungal colonization or infection participates in the pathogenesis of destructive periodontal disease (11, 29).

Early in the AIDS epidemic, some studies found that the microflora of oral subgingival sites includes *C. albicans* in as many as 50 to 60% of diseased sites of AIDS patients (10, 25, 26, 32). It was suggested that when *C. albicans* gains access to underlying periodontal tissues, damage results from the metabolites produced by the yeast (10). Yeast species in the subgingival tissue of AIDS patients may also be the source of disseminated candidosis. The most recent study performed by Hannula et al. (11) on isolation of subgingival yeasts reported that the mean proportion of total microbiota was higher for *C. albicans*-positive samples than for *C. albicans*-negative samples from selected subjects, indicating the need for further analysis to determine the relationship between subgingival occurrence of *C. albicans* and periodontal diseases.

C. dubliniensis has been shown to have, in addition to greater adherence to human buccal epithelial cells, mucin, and the oral bacterium *Fusobacterium nucleatum*, significantly higher proteinase activity, which was shown to degrade mucins, the major constituents of mucus (7, 15, 16, 20, 21). With interactions among microorganisms shown to be an important step in infectious disease processes in the oral cavity, the ability of *C. dubliniensis* to adhere to *F. nucleatum* may aid in colonization of deep sulci of periodontal pockets (6, 8, 19).

In this investigation, 44 of the 54 patients sampled were positive for fungus, with 21 (48%) positive for *C. dubliniensis*. Of the 44 positive patients, 29 (66%) had positive subgingival cultures; 15 (39%) of these samples were positive for *C. dubliniensis*. In addition to *C. dubliniensis* and *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *S. cerevisiae* were the other species recovered from the subgingival samples (Table 1). In contrast, results of fungal cultures of the control, HIV-negative group demonstrated one patient with a subgingival culture of light growth of *C. albicans*. No other species were recovered.

This study reports, for the first time, the recovery of *C. dubliniensis* from subgingival periodontal lesions and confirms the presence of *Candida* in the subgingival sites of HIV-positive patients with periodontal diseases. The high frequency of recovery of *Candida* from subgingival samples of immunocompromised individuals, in comparison to that of a control group, supports the need for further investigations to determine the role that *Candida* species, and in particular *C. dubliniensis*, may play in the progression of periodontal lesions.

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