The detection of oral *Candida* in pediatric leukemia patients

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Abstract

Among leukemia patients, a significant number of deaths are due to Candida septicemia, many of which are associated with previous oral infections. Oral candidiasis detection methods vary, and the relationship between oral candidiasis and Candida colonization (CC) is not well defined. The main objectives of this study were to compare the incidence of CC in a healthy and leukemic population, and also to evaluate the efficacy of three simple and inexpensive methods of detecting oral CC in predicting the occurrence of oral candidiasis. A secondary objective was to portray speciation in the examined populations. Forty-two pediatric leukemia patients and 42 healthy, age-, race-, and gender-matched control patients participated in this study. The three methods of detection were cytological examination of the oral mucosa, and direct culture methods from mucosal smears using Sabouraud's dextrose agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) and Oricult-N (Orion Diagnostica, Espoo, Finland). This study demonstrated an increased prevalence of CC in pediatric leukemia patients with the direct culture method detecting CC in a significantly greater proportion of the population (Oricult-N, P = 0.034; Sabouraud's dextrose agar, P = 0.0036). Candida albicans was the predominant species. Further study is needed to determine the clinical significance of oral CC and its relationship to oral candidiasis and systemic infection in pediatric leukemia patients. (Pediatr Dent 14:236–39, 1992)

Introduction

Although *Candida* is a commensal inhabitant of oral mucosal surfaces in approximately 50% of healthy individuals, some diseases and therapies compromise host defenses, allowing *Candida* to become a factor in treatment morbidity that can even become life threatening.[9, 17] Conditions which are favorable for oral candidiasis include endocrine disorders, immunodeficiency states, and use of broad-spectrum antibiotics, corticosteroids, and antineoplastic drugs.[7] Leukemia patients undergoing chemotherapy present an optimal environment for the development of oral candidiasis, especially during periods of neutropenia. *Candida* species are responsible for approximately one-half of all oral infections occurring during antileukemia chemotherapy.[8]

The diagnosis of oral candidiasis is initially based on the patient's medical status and clinical findings. The form of oral candidiasis most commonly found in leukemia patients is acute pseudomembranous candidiasis which often appears as white plaques on the mucosa of the tongue, cheeks, gingiva, and pharynx.[7, 8] However, infection may occur without the appearance of white plaques, and confirmation of the clinical diagnosis may be accomplished with supplementary procedures, such as direct cultures and exfoliative cytology.[19]

The most common technique used for diagnosis of oral candidiasis is microscopic examination of exfoliative cytology from tongue and buccal mucosa. Cytologic smears are stained using the periodic acid-Schiff's (PAS) method, Gram's stain or wet mount preparation in 10% KOH. Smears which demonstrate hyphal forms are considered diagnostic of oral candidiasis.[5, 20] However, clinical judgment is still needed in diagnosis since hyphal forms can be found in the absence of significant numbers of inflammatory cells.[2, 17] While this technique is time-efficient and reliable, speciation cannot be accomplished without a culture.[3, 5, 7, 13, 14, 19]

Numerous techniques utilizing the direct culture method exist. Oral swabbing or salivary samples may be cultivated on prepoured plates or slides of a media suitable for fungal growth, such as Sabouraud's dextrose agar and Nickerson's media.[4, 16] Since *Candida* is an important fungus in human disease, several specialized culture methods have been developed for its rapid identification. One system is Oricult-N (Orion Diagnostica, Espoo, Finland), which uses a modification of Nickerson's media that selects for *Candida albicans*.[3] From direct cultures, *Candida* organisms can be identified and speciated on the basis of morphologic characteristics and assimilation tests.[2]

The detection of oral *Candida* colonization (CC) is important in the treatment of leukemia patients, since the oral cavity may serve as a reservoir for systemic infection.[12] While children with leukemia are surviving longer due to advances in the diagnosis and treatment of the disorder, they now have a greater risk of developing opportunistic infections, such as candidiasis, during antineoplastic therapy. The frequency of an infectious *Candida* complication in pediatric oncology patients has been reported as high as 38%.[6]

The identification of risk factors for the development of candidiasis, such as the detection of oral *Candida*, is a prerequisite for evaluating and instituting preventive measures to decrease morbidity in children during cancer therapy. The first purpose of this investigation was to compare the incidence of CC in a healthy and leukemic population. The second purpose was to evaluate the efficacy of three simple and inexpensive methods of detection (exfoliative cytology and direct culture on Sabouraud's and Nickerson's media) of oral *Candida* in predicting the development of oral candidiasis. As a point of interest, speciation of the examined populations also was completed.

Table 1. Age, gender, and race distribution

Group	*Total*	*Age Range*	*Male*	*Female*	*Caucasian*	*Black (Indian)*
ALL*	39	7M — 16Y	23	16	31	7 (1)
ANLL†	3	8Y — 12Y	1	2	3	0
Control	42	1Y — 16Y	24	18	34	7 (1)

* Acute lymphoblastic leukemia

† Acute nonlymphocytic leukemia

Table 2. Leukemia patient status

*Patient Status**	*Induction Therapy*	*Maintenance Therapy*	*Neutropenia and Fever*	*Follow-up*
Hospitalized	9	25	11	0
Outpatient	6	46	2	10

* 109 separate visits to 42 patients over six months

Table 3. Per cent of leukemia group receiving drug therapy

Cytotoxic	*Antibiotic*	*Steriod*	*Antifungal*
66.1%	70.6%	21.1%	5.5%

Materials and Methods

Forty-two pediatric leukemia patients were examined at initial diagnosis, during therapy, or during follow-up evaluation. The disease diagnosis, age, gender, and race distributions of the two groups are presented in Table 1. Data collection varied in the leukemic group, depending on the time of diagnosis and phase of therapy. Patients were examined while hospitalized (e.g., for chemotherapy or fever with neutropenia), or during outpatient therapy (e.g., for chemotherapy or follow-up visits, Table 2). One hundred and nine oral examinations were performed, representing one to three examinations for each patient. After informed consent, a thorough medical and dental history was obtained from each patient upon enrollment in this study and updated at each visit. The specific diagnosis, treatment protocol, medications, nonoral systemic complications, platelet count, and differential white cell counts were obtained by a thorough review of hospital records. In addition, age-, gender-, and race-matched controls were selected from children with no significant medical histories, as determined from a health questionnaire.

Each patient received an oral examination using a tongue blade and artificial light. Mucositis, ulcerations, and white plaques were rated by area and size.

Mucosal samples were obtained from all leukemia and control patients at each session. For cytological screening, a sterile tongue blade was used to rub the buccal mucosa and the dorsal and ventral surfaces of the tongue firmly. The collected material was applied and fixed to a microscope slide. The specimen was stained using the PAS method and evaluated microscopically to determine the degree of CC by rating: 0, negative; 1, carrier state; 2, minimal colonization; 3, extensive colonization, as previously described.[20]

For the direct culture methods, specimens were collected by gently rubbing a sterile cotton swab over the buccal mucosa and the dorsal and ventral surfaces of the tongue. Using the Oricult-N system as described by Orion Diagnostica (Espoo, Finland), an Oricult-N dip slide was inoculated immediately by rolling the swab over the slide.[3] A second swab was used to inoculate a plate of Sabouraud's dextrose agar. The Oricult-N tube and the Sabouraud plate were sealed lightly and incubated at 37°C for two days. The degree of colonization was defined by the number of fungal colonies on the selected media, which was rated: 0, no growth; 1, sparsely colonized (1–20 colonies); 2, abundantly colonized (confluent growth). To confirm *Candida*, all fungal growth was speciated on the basis of germ tube tests and assimilation tests.[2]

The examination data were tabulated and analyzed. Statistical correlation of CC and oral lesions was determined through the use of Pearson correlation coefficient. Fisher's two-tailed exact tests and Chi-square tests were used to test for significant differences in CC between the control group and leukemia group. Fisher's two-tailed exact test (with results classified as 0 = negative fungal growth and 1 = positive fungal growth) was used to test for significant differences in the methods of detecting CC. Statistical significance was accepted at $P < 0.05$.

Results

Forty-two leukemia patients with gender-, age-, and race-matched controls were enrolled in this six-month study. Thirteen patients were examined during periods of fever with neutropenia, and three had blood cultures positive for bacteria. There were no blood cultures positive for fungal organisms during the six months of this study.

The drug therapy for each patient depended on the course of the leukemia and the antileukemia therapeutic protocol. The major drug groups were cytotoxic, antibiotic, steroid, and antifungal drugs (Table 3, see previous page). The only antifungal drug used during this study was Nystatin, which was administered to patients with oral candidiasis. Some patients with oral candidiasis also rinsed with chlorhexidine. Antibiotic therapy was the only drug regimen to show significant correlation with CC ($P = 0.0163$).

In the leukemia group, fungi were detected in 23 of 97 (24%) of cytological smears, 38 of 109 (35%) of Oricult-N cultures, and 40 of 105 (38%) of Sabouraud cultures. In the control group, fungi were detected in two of 41 (5%) of cytological smears, four of 42 (10%) of Oricult-N cultures, and five of 42 (12%) of Sabouraud cultures. The number of leukemia patients who presented with oral CC was significantly greater than the number of control patients by all methods of detection ($P = 0.004$, Figure).

When compared to exfoliative cytology, the direct culture methods detected CC in a significantly greater proportion of the population (i.e., Oricult-N, $P = 0.034$; Sabouraud's dextrose agar, $P = 0.0036$). The difference between the direct culture methods was not significant ($P = 0.064$).

Eighteen oral lesions were found in seven leukemia patients. The lesions consisted of mucositis (2), white plaques (7), and/or mucosal ulcerations (9). Of the seven leukemia patients demonstrating oral lesions, five had cytological smears positive for fungi, and six had cultures positive for fungal growth. Using all three methods of detection, the occurrence of oral white plaques correlated strongly with CC ($P = 0.0295$), while mucosal ulceration was not correlated significantly with CC ($P = 0.0683$). No individual technique of detection showed significant correlation with oral lesions.

Speciation with germ tube and biochemical assimilation tests found that all but three of the oral fungal cultures were *C. albicans*. The remaining cultures were identified as *C. tropicalis* (2) and *Rhodotorula rubra* (1).

Discussion

Disease and treatment factors of leukemia cause changes in the oral cavity, resulting in the proliferation of oral *Candida*.[5, 20] This study found that a significantly

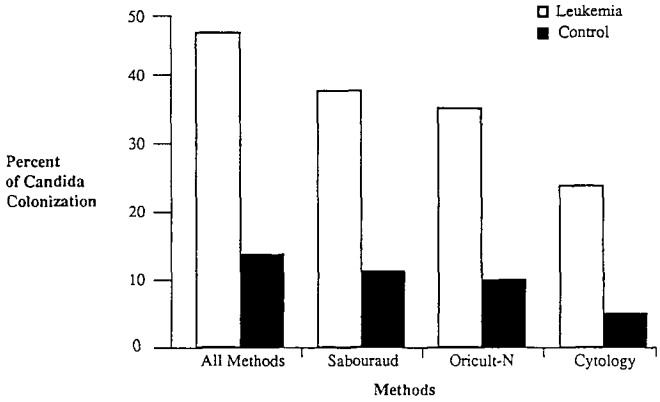

Figure. Presence of oral *Candida* colonization as detected by each method.

greater percentage of the pediatric leukemia group (46%) exhibited oral CC when compared with the healthy control group (14%). This agrees with previous adult studies of acute leukemia populations, which reported oral CC in approximately 50% of leukemia patients using direct culture and cytological examination as the detection methods.[7, 9]

When interpreting results of studies regarding the incidence of oral *Candida*, the method of detection must be considered. The least reliable method is the observation of clinical lesions, since infection often may be present without any sign or symptom. While the fungal organisms were present in the oral cavity of 46% of the leukemia patients in this study, only 15% of the group presented with oral signs of fungal infection. This finding is similar to a previous study of a pediatric leukemia population in which the reported incidence of CC was 21%, with candidiasis defined as the presence of oral lesions.[15] Another study of 50 adult leukemia patients detected CC with cytological examination in approximately 90% of the patients. During the course of the study, invasive oral colonization developed in 30 patients and *Candida* sepsis developed in nine patients, but only three cases of clinical candidiasis were observed.[19]

Exfoliative cytology is often the preferred method of *Candida* detection, since it is considered to differentiate the healthy carrier state from oral candidiasis by the presence of hyphal forms. In the present study, the incidence of oral *Candida* in the leukemia group was 24% using cytology. Twelve slides were not evaluated due to the absence of adequate numbers of epithelial cells in the smears. Six of the 12 inadequate slides were taken from patients younger than 5 years of age, and three of the remaining six were taken from patients who were hospitalized for fever with neutropenia. Therefore, age and degree of debilitation may play a role in the examiner's ability to acquire adequate mucosal

scrapings for exfoliative cytology.

While not as time-efficient as exfoliative cytology, direct culture is a simple and reliable method of detecting oral CC. The incidence of oral CC in this study was 35% by Oricult-N and 38% by Sabouraud's dextrose agar. A criticism of the culture technique is that in the absence of clinical lesions, a definite diagnosis of oral candidiasis cannot be made. However, specialized culture methods exist, such as imprint culturing, which quantitate CC and differentiate carrier state from infection.[1, 10] While imprint culturing would be a more complete method for detecting CC, it would not be cost effective to use this method routinely in a hospital setting.

The greatest advantage of all the culture techniques over exfoliative cytology is the ability to speciate organisms. Speciation of the fungal cultures revealed all but three cultures to be *C. albicans*,, which agrees with previous research citing *C. albicans* as the most commonly cultured *Candida* species from the oral cavity.[1, 21] Of the remaining three cultures, two were *C. tropicalis*, which is commonly found as an oral inhabitant of leukemia patients and has been associated with *Candida* sepsis.[9, 19] The remaining culture was *R. rubra* which is infrequently isolated from oral cultures of leukemia patients, but has been reported to cause septicemia in association with contaminated catheters.

Conclusion

Children with leukemia have an increased incidence of oral colonization by *C. albicans* , compared with healthy children. Although both cytologic and direct culture examination could help in diagnosing oral candidiasis when lesions occur, this study did not find the detection of CC to be of clinical importance in predicting oral candidiasis. Other clinical findings such as antibiotic therapy are possibly as efficient in predicting CC, especially on a cost-effective basis. Further study with more extensive follow up is required to evaluate the importance of CC in oral and systemic complications.

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The procedures, possible discomforts or risks, as well as possible benefits were explained fully to the human subjects involved, and their informed consent was obtained prior to the investigation.

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