

## Oral colonization of *Candida* species in perinatally HIV-infected children in northern Thailand

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**Abstract:** Human immunodeficiency virus (HIV) infection remains a serious problem in northern Thailand. A high prevalence of perinatally HIV-infected children with oral candidiasis has been observed in the region. The objective of this study was to determine oral colonization of *Candida* spp. in children with perinatal HIV infection. Samples were collected by oral rinse or oral swab from 40 HIV-infected children and from 15 HIV-negative children as a control group. Yeasts recovered in culture were identified and quantified. The mean ages of HIV-infected children and HIV-negative children were 5.5 years (SD = 3.5) and 2.9 years (SD = 2.0) respectively. Eighteen HIV-infected children (45%) had clinical symptoms of oral candidiasis while none of the HIV-negative children had any such symptoms. By culture technique, yeasts were isolated from 28/40 (70%) of the HIV-infected children and 6/15 (40%) of the HIV-negative children. *C. albicans* was the most common species recovered from HIV-infected and HIV-negative children. Statistically, HIV infection was significantly associated with *Candida* spp. detection ( $P$ -value = 0.04). In contrast, the association between HIV infection and asymptomatic oral carriage of *Candida* spp. was not significant ( $P$ -value = 0.74). These findings demonstrate that oral colonization of *Candida* spp. is prevalent in HIV-infected children and suggest that prevention and treatment of oral candidiasis is needed for these children. ( J. Oral Sci. 46, 101-105,

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Key words: human immunodeficiency virus infection; perinatal; children; oral candidiasis; *Candida albicans*.

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### Introduction

Human immunodeficiency virus (HIV) infection remains a serious health problem in Thailand, especially among children. Approximately 98% of HIV-infected children in developing countries have acquired HIV from their mothers during pregnancy, at delivery or through breastfeeding (1). Reportedly, the number of HIV-infected women and children is increasing at an alarming rate in the Asia-Pacific region (2).

Oral lesions are prevalent among HIV-infected children (3,4). Of all oral lesions, candidiasis is the most common in these children. *Candida* spp. are opportunistic yeast-like fungi. *C. albicans* is by far the most common species isolated from oral candidiasis. Although *Candida* spp. can be carried as an oral commensal in healthy individuals, several local and systemic factors including HIV infection can predispose these individuals to oral candidiasis (5). The few reports investigating the oral colonization of *Candida* spp. in HIV-infected children have yielded variable results, probably due to the influence of geographic and ethnic differences (6). However, there are no data relating to Thai children. Taken together, a lack of information about the oral colonization of *Candida* spp. in Thai children prompted us to conduct a study in 40 perinatally HIV-infected children in comparison with 15 HIV-negative children.

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## Materials and Methods

### Subjects

Forty perinatally HIV-infected children and 15 HIV-negative children who had HIV-infected mothers were recruited from the pediatric HIV outpatient department, Nakornping Provincial Hospital, Chiang Mai, Thailand during January-March 2002. The age of the HIV-infected children ranged from 1.5 to 12 years with a mean of 5.5 years (SD = 3.5). There were 18 boys and 22 girls. The mean age of HIV-negative children was 2.9 (SD = 2.0), ranging from 0.5 to 7 years (6 boys and 9 girls). Consent to oral examination and sample collection for research purposes was given by the parents or guardians of all children. This project was approved by the Human Subject Protection Committee, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand. All HIV-infected children were diagnosed by repeated enzyme-linked immunosorbent assay (ELISA), ELISA with Western blot confirmation test and/or repeated polymerase chain reaction (PCR). Diagnosis of oral candidiasis was based on clinical appearance according to the classification and diagnosis for orofacial lesions in HIV-infected children, proposed by Ramos-Gomez et al (7).

### Sampling

The samples were collected by an oral rinse method (8). Briefly, a 10-ml sample of sterile phosphate-buffered saline (PBS) was swirled in the mouth for 1 min, then expelled into a container which was sent to the laboratory for determination of fungal pathogens. The resultant growth was quantified. In uncooperative young children who were unable to rinse, samples were obtained by taking oral swabs from the dorsal surface of the tongue. These swabs were immediately immersed in PBS and taken to the laboratory for culture. Sixteen oral rinses and 24 oral swabs were taken from the HIV-infected children. Five oral rinses and 10 oral swabs were taken from the HIV-negative children.

### Culture and identification of isolates

The oral rinses were centrifuged at  $1,700 \times g$  for 10 min. The pellet was resuspended in 1.0 ml of PBS, and vortexed for 30 s. The number of fungi in each suspension was determined using a dilution spread plate on Sabouraud's dextrose agar (Difco Laboratories, USA). Plates were incubated for 48 h at 37°C. The number of colony-forming units (CFU) for each sample was quantified. Oral swabs were inoculated on Sabouraud's dextrose agar plates (Difco Laboratories, USA). Plates were streaked and incubated for 48 h at 37°C. The amount of yeast was semiquantified and graded into no growth, light, moderate or heavy

growth. The organisms and species were identified based on the following criteria: colony and microscopic morphology, germ tube test, chlamydoconidia formation test, hydrolysis of urea, sugar assimilation and fermentation tests (9).

Statistically, the association between HIV infection and *Candida* spp. detection, and that between HIV infection and asymptomatic oral carriage of *Candida* spp. were analyzed using the Pearson chi-squared test, with a significance level of  $P < 0.05$ .

## Results

In the HIV-infected group, clinical oral candidiasis was found in 18 children (45%). Of these children, 13 exhibited the pseudomembranous type, 10 the erythematous type, and 4 exhibited angular cheilitis. Yeasts were isolated in 28/40 (70%) (Table 1), ranging from light to heavy colony growth in the swab technique samples, and 330 to  $1.0 \times 10^5$  CFU/ml in the oral rinse samples. The asymptomatic oral carriage rate of *Candida* spp. in HIV-infected children was 45.5% (Table 2). The identification of *Candida* spp. is shown in Table 3, revealing that *C. albicans* was the most commonly isolated yeast (26/28 cases). The other two isolates were *C. glabrata* and *C. krusei*.

None of the HIV-negative children had clinical symptoms of oral candidiasis. Yeasts were isolated from 6/15 (40%) (Table 1), ranging from light to moderate colony growths in the swab technique samples and 170 CFU/ml from an oral rinse sample. *C. albicans* was the most common species (5/6 cases); the only other was *C. tropicalis* (Table 3).

The association between HIV infection and *Candida* spp. detection was statistically significant ( $P = 0.04$ ). On the other hand, the association between HIV infection and asymptomatic oral carriage of *Candida* spp. was not statistically significant ( $P = 0.74$ ).

## Discussion

The present study analyzed the prevalence, quantity and type of species of *Candida* in perinatally HIV-infected children in comparison with HIV-negative children. To our knowledge, this is the first study to investigate the oral colonization of *Candida* spp. in HIV-infected children in Asia. We found that candidal colonization was more prevalent in HIV-infected (70%) and symptomatic HIV-infected children (100%) when compared with the asymptomatic HIV-infected (45.5%) and HIV-negative groups (40%). These findings were consistent with a previous study from the USA where 50% of HIV-positive and 30% of non-HIV-positive children were positive for the fungi (6). Similarly, a study by Hicks et al. (1998)

Table 1: Prevalence of clinical candidiasis and *Candida* spp. detection

Group	n	n (%)	n (%)
		clinical candidiasis	yeast detected
HIV-infected children	40	18 (45)	28 (70)
HIV-free children	15	0 (0)	6 (40)

Table 2: Asymptomatic oral carriage of *Candida* spp.

Group	n	n (%)
		asymptomatic oral carriage
HIV-infected children	22	10 (45.5)
HIV-free children	15	6 (40.0)

Table 3: *Candida* spp. identification

<i>Candida</i> spp.	n (%)	n (%)	n (%)	n (%)
	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>	<i>C. krusei</i>
HIV-infected children	26	0	1	1
HIV-negative children	5	1	0	0

demonstrated that *Candida* spp. was cytologically detectable in the saliva of 19% of HIV-infected and 9% of HIV-exposed, but uninfected children (10). The differences in the percentage of positive cases in our study and in previous studies may be due to geographic variation, time of sampling and techniques used for detection (11). Of all available techniques, the oral rinse method is accepted as being superior to other sampling techniques, for example imprint culture, swabbing and cytological techniques (8,10). In our study, we also found a high incidence of *Candida* spp. in symptomatic HIV-infected children (100%) while it was detected in only 45.5% of asymptomatic HIV-infected children. The different results between these two groups may be due to the fact that the entire group of symptomatic HIV-infected children showed clinical manifestations of candidiasis. Recently, it was found that malnutrition in children may also contribute to the high frequency of yeasts and yeast species other than *C. albicans*

(6). Data from adults have also revealed that oral colonization of *Candida* spp. was prevalent in HIV-infected individuals in Thailand (66.7%) (12), Hong Kong (54.8%) (13) and Germany (65.6% - 73.8%) (14,15).

Among *Candida* spp. recovered from HIV-infected and HIV-negative children in our study, *C. albicans* was the most common species. The other species, only rarely isolated, were *C. tropicalis*, *C. glabrata*, and *C. krusei*. These results were in line with previous studies of HIV-infected children (6) and HIV-infected adults (12). In normal individuals, *C. albicans* is a harmless commensal of the oral mucosa. However, in patients with immunosuppressed conditions, particularly HIV infection, the disease can progress to oropharyngeal candidiasis, and can disseminate and eventually be fatal (16). Many previous investigations have delineated the molecular mechanisms responsible for the virulence of *Candida* spp. and found that phenotypic switching affects a variety of

virulence traits of the organism including the bud-hypha transition, antigenicity, adhesion, sensitivity to neutrophils and oxidants, secretion of proteinase and drug susceptibility (17). Most strains of *C. albicans* and related species are capable of phenotypic switching spontaneously, reversibly, and at high frequencies. Furthermore, the average strain of *C. albicans* colonizing the oral cavity of HIV-positive individuals prior to the first episode of oral thrush and prior to anti-fungal therapy has been found to be already in a high-frequency mode of phenotypic switching and already more resistant to a number of common anti-fungal agents than the average commensal strain colonizing healthy individuals (18). Collectively, these findings suggest that HIV-positive children have a higher chance of developing anti-fungal drug resistance than HIV-negative individuals.

In our study, we found that the asymptomatic oral carriage rate of *Candida* spp. in HIV-infected children was 45.5% while that of HIV-negative children was 40%. By using cytological analysis, 11% of asymptomatic HIV-infected children were identified as asymptomatic carriers (10). Previous studies have shown a wide range of oral carriage rates of *Candida* spp. in HIV-infected (11%–96%) and healthy adults (10%–68%) (19). Again, the variation in these findings was suggested to be due to geographic differences, time of sampling or different methods for yeast recovery and quantification (11). Early detection of oral carriage of *Candida* spp. is seen to be important in initiating anti-fungal treatment prior to overt oropharyngeal candidiasis. In addition, detection of oral carriage may also identify children with the propensity for rapid progression of HIV infection since oral carriage may influence the development of clinically significant candidiasis in these immunocompromised children (10). Thus, long-term follow-up of carriers with HIV infection is especially crucial.

In conclusion, oral colonization of *Candida* spp., especially *C. albicans*, was detected with high frequency in HIV-infected children. A high rate of oral carriage was also observed in this group.

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