ORAL DISEASES

Oral Diseases (2011) 17, 393–398 doi:10.1111/j.1601-0825.2010.01765.x

www.wiley.com

ORIGINAL ARTICLE

Oral colonization by *Candida* species in AIDS pediatric patients

C Domaneschi[1], DB Massarente[2], RS de Freitas[3], HH de Sousa Marques[4], CR Paula[5], DA Migliari[6], JLF Antunes[7]

[1]*PhD, Department of Oral Diagnosis, School of Dentistry, University of São Paulo, Brazil;* [2]*Researcher fellow, Department of Social and Preventive Dentistry, School of Dentistry, University of São Paulo, Brazil;* [3]*Micologist, Laboratory of Mycology, Institute of Tropical Medicine, University of São Paulo, Brazil;* [4]*MD, PhD, Child's Institute, School of Medicine, University of São Paulo, Brazil;* [5] *Professor, Department of Micology, Biomedical Institute of Science, University of São Paulo, Brazil;* [6]*Professor, Department of Oral Diagnosis, School of Dentistry, University of São Paulo, Brazil;* [7]*Professor, Department of Epidemiology, School of Public Health, University of São Paulo, Brazil*

The aim of this study was to assess the prevalence of factors associated with oral colonization by *Candida* spp. in pediatric patients with AIDS. The sample comprised of 117 children. Clinical status, medicines in use, and laboratory findings were obtained from hospital records; sociodemographic data were given by relatives. A dental examination assessed the prevalence of dental caries. The prevalence of oral colonization by *Candida* was 62%. Only seven children presented clinical manifestation of oral candidosis despite their high viral load index and low-for-age CD4 count. *Candida* colonization was directly associated with frequent use of antibiotics (prevalence ratio [PR] = 1.44), sulfa drugs (PR = 1.23), alteration in the oral mucosa (PR = 1.55), and untreated dental caries (PR = 1.93). It was inversely associated with the use of antiretroviral therapies (PR = 0.65). *Candida albicans* was the most frequently detected species (80%); phenotypic tests did not detect *C. dubliniensis* strains. This study observed a low prevalence of *Candida*-related oral lesions in these patients, which is compatible with the hypothesis that antiretroviral medicines may have contributed to reducing oral manifestations from *Candida* infection. The high prevalence of *Candida* colonization in HIV+/AIDS children with untreated dental caries reinforces the importance of oral health care in interdisciplinary health units that assist these patients.

Oral Diseases (2011) **17**, 393–398

Keywords: *Candida* spp; HIV infection; AIDS; dental caries; sociodemographic condition

Correspondence: Carina Domaneschi, Universidade de São Paulo, Faculdade de Odontologia, Departamento de Estomatologia, Av: Prof. Lineu Prestes, 2227-Cidade Universitária, São Paulo, SP, Brazil 05508-900. Tel/Fax: +55 -11-3091 7893, E-mail: domaneschi@usp.br
Received 26 November 2009; revised 16 August 2010; accepted 2 September 2010

Introduction

More than two million children younger than 15 years of age are estimated to be infected by the human immunodeficiency virus (HIV). The acquired immunodeficiency syndrome (AIDS) is one of the leading causes of death among children worldwide (UNAIDS, 2004). Most children infected by HIV manifest the initial symptoms of AIDS before their first birthday, and oral manifestations are the first signs of the disease for nearly half of them (European Collaborative Study, 1991). More than 40 types of oral diseases have been described as being highly prevalent in these patients (Hoare, 2003; Miziara *et al*, 2006).

The detection of opportunistic infections such as oral candidosis is indicative of poor prognosis for HIV-infected patients. The early recognition of oral signs associated with AIDS may facilitate therapeutic interventions aimed at reducing the clinical impact of the disease. In addition to the overall improvement of survival and quality of life, the introduction of new antiretroviral drugs reduces the frequency of some oral manifestations in HIV-infected adult patients (Cauda *et al*, 1999). However, the effect of antiretroviral medicines on oral diseases in AIDS pediatric patients has been scarcely investigated (Kozinetz *et al*, 2000).

Oral candidosis is caused by *Candida* spp., predominantly by *Candida albicans*. Although less prevalent, *C. dubliniensis*, originally described in 1995, has been identified as an important etiologic agent, owing to its pathogenic characteristics, resistance to antifungal agents, and greater adhesion to cells of the oral mucosa (Alves *et al*, 2002; Arikan *et al*, 2003). However, *C. dubliniensis* and *C. albicans* present similar phenotypic and genotypic characteristics, and the precise identification of the yeast is relevant to the planning of treatment protocols. Few studies have assessed the prevalence of the infection caused by *C. dubliniensis* in pediatric

patients with AIDS (Brow *et al*, 2000; Cimolai *et al*, 2002).

The present study aimed to assess the prevalence of oral colonization by *Candida* spp. in pediatric patients affected by AIDS and to identify the socioeconomic, behavioral, and clinical factors associated with this outcome. This study also aimed at specifically assessing yeast incidence of *C. dubliniensis* in these patients.

Materials and methods

Clinical samples

The sample comprised of 117 children with AIDS (aged 3–15 years; mean age, 9.4) enrolled in the outpatient unit of the Child's Institute, School of Medicine, University of São Paulo, Brazil (ICR/HC). AIDS was positively diagnosed for all children participating in the study, that is, in addition to being infected by HIV (vertical transmission), these children already manifested AIDS symptoms and/or had a low-for-age CD4+ count (Centers for Disease Control Prevention, 1994).

Ethical clearance was given by the Committees on Ethics of participating Institutions (School of Medicine and School of Dentistry, University of São Paulo); parents (or legal representatives) signed the informed consent form.

Mycological exam

The oral mucosa of all patients, with or without clinical manifestation of oral candidosis (erythematous or pseudomembranous), was examined, and microbiologic samples of the oral mucosa were obtained using a sterile cytobrush. Swabs were inoculated in agar sabouraud-dextrose (ASD) medium.

Direct microscopy examination

This study considered oral colonization by *Candida* spp. when the presence of yeasts was exclusively assessed by direct examination (using 10% KOH); whereas oral infection by *Candida* spp. was considered when, in addition to yeast form, there was the presence of pseudohyphae or hyphae. The ability to germinate and form hyphae may be a factor for the virulence of this organism *in vivo*. With regard to *C. glabrata*, the quantity of yeast forms was taken into consideration because this species does not produce pseudohyphae and hyphae. The culture was considered positive when there was growth of colonies in the ASD medium.

Yeasts were identified macroscopically and microscopically in terms of reproductive and physiologic characteristics based on the book of Hoog *et al* (2000). All samples were cultivated in ASD medium for 24 h at 37°C before being assayed, and were kept in the mycology section of the Microbiology Department in the Biomedical Sciences Institute, University of São Paulo, Brazil. The identification of these yeasts was performed in two mycological laboratories in the University of São Paulo (Institute of Tropical Medicine and Institute of Biomedical Sciences, University of São Paulo, Brazil).

Reference strains

For phenotypic differentiation between *C. albicans* and *C. dubliniensis*, reference strains *C. albicans* (ATCC 64548) and *C. dubliniensis* (ATCC 777) were used.

Identification tests

Presumptive identification of *C. albicans* was performed by the germ-tube test. The organisms that failed to form a germ tube following a 3-h incubation at 37°C in sterile bovine fetal serum were analyzed. *Candida* spp. was classified and identified on the basis of a limited number of morphologic features and biochemical properties such as ability to produce true hyphae, pseudohyphae or chlamydospores, and ability to assimilate a range of compounds such as the sole source of carbon or nitrogen. Although morphology may contribute to identification, biochemical tests are demanded for an appropriate diagnosis (Hoog *et al*, 2000).

To differentiate *C.albicans* from *C. dubliniensis*, samples identified as *C. albicans* were assessed in terms of colony color on chromogenic medium, (CHROMagar – CHROMagar^{tm} Candida, Paris, France). In this medium, colonies of *C. albicans* are light green, whereas colonies of *C. dubliniensis* are dark-green (Sullivan and Coleman, 1998), and growth in ASD medium at 45°C were in accord with Pinjon *et al* (1998), Kurzai *et al* (2000) and Giammanco *et al* (2002); they verified the thermotolerance test, which has been used to distinguish isolates of *Candida* species. Those authors observed that *C. dubliniensis* did not grow on agar media at the incubation temperature of 45°C. The thermotolerance test at 45°C, together with the assimilation test (xylose and trehalose), is able to differentiate *C. dubliniensis* from *C. albicans* properly. *C. albicans* is able to assimilate xylose and trehalose whereas *C. dubliniensis* does not have this ability (Pincus *et al*, *1999;* Ahmad *et al*, 2004; Al-Sweih *et al*, 2005).

Sociodemographic information, clinical status, and prevalence of dental caries

Family caregivers answered a detailed questionnaire on sociodemographic characteristics. The form also comprised of a section that determined behavioral characteristics of patients, the frequency of brushing their teeth and flossing, and the number of dental check-up visits.

One of the authors (CD) performed oral examinations for the diagnosis of dental caries using the international criteria standardized by the World Health Organization for oral health surveys (World Health Organization, 1997). Patients were examined in hospital under natural light, using gloves, plane mouth mirrors, and periodontal (CPI) probes. Untreated dental caries were considered prevalent in patients with at least one permanent or deciduous tooth with decay.

Information regarding the use of drugs, specifically referred to as the continuous use of antiretroviral drugs and antimicrobial agents, and the general clinical status were obtained from hospital records of the patients. Severity of symptoms was based on the (Centers for Disease Control Prevention, 1994)-revised HIV pediatric classification system. Viral load is the number of

HIV-RNA copies per milliliter of plasma. The cutoff (100 000 copies per ml) delimits a higher long-term risk for death in HIV-infected children. CD4+ T-lymphocyte count was classified as low-for-age, according to limits evidencing moderate or severe immunologic suppression as follows: ≤1000 cells mm^{-3} (1–5 years old), ≤500 (6–12 years), and ≤350 (13 years or more) (Centers for Disease Control Prevention, 1994).

Data analysis

For statistical analysis, we used Stata 8.0, version 2003. The association between oral colonization by *Candida* and covariates on sociodemographic characteristics, dental status, and continuous use of medicine by patients was appraised in terms of prevalence ratio, as assessed by Poisson regression analysis (Barros and Hirakata, 2003), with robust variance estimation and control, by age. The fitting of a multivariate model of Poisson regression analysis considered the adjustment of the outcome to covariates selected by a backward stepwise procedure, with $P < 0.25$ as the cutoff for entering and $P < 0.05$ for keeping covariates in the model.

Results

In all the 117 children, oral colonization by *Candida* yeasts was prevalent in 62% (54–72%, a 95% confidence interval), and oral candidosis was present in only seven children.

For each child, we collected one sample representing the whole extension of the oral mucosa. We also collected 3 additional samples related to the tongue, and 4 related to the soft palate for 7 children that manifested clinical signs of *Candida* colonization in these specific sites, thus totalling 124 samples. The culture showed positive colonization by *Candida* in 86 samples of the total. Of the 86 samples of yeasts isolated, the most frequent species were *C. albicans* (69/86), followed by *C. tropicalis* (5/86), *C. kefyr* (4/86), *C. krusei* (3/86), *C. glabrata* (2/86), and *C. guilliermondii* (1/86). Two samples were not identified due to growth, slower than that of other species. The frequency of the growth of different species of *Candida* isolated from oral mucosa of patients with AIDS is shown in Figure 1.

Sixty-nine samples identified as *C. albicans* were assessed in terms of growth on chromogenic medium and ASD medium at 45°C to differentiate them from *C. dubliniensis*. Of these samples, 14 were suggestive of *C. dubliniensis* in chromogenic medium, as they turned into dark-green color. However, the thermotolerance test showed that all 14 samples grew well at 45°C. In addition, all these samples were positive to the assimilation tests for xylose and trehalose, thus characterizing these strains as *C. albicans*. In this study, no strain of *C. dubliniensis* was identified.

The demographic data and behavior analysis of 117 studied-children are shown in Table 1. Sixty-three of them were females; seventy-three were blacks (no distinction between lighter and darker skinned blacks). There were positive results in culture examination associated with untreated dental caries (PR = 1.86; 1.28–2.69) and having more than two siblings (PR = 1.42; 1.06–1.90). The caregivers had less than 11 years at school.

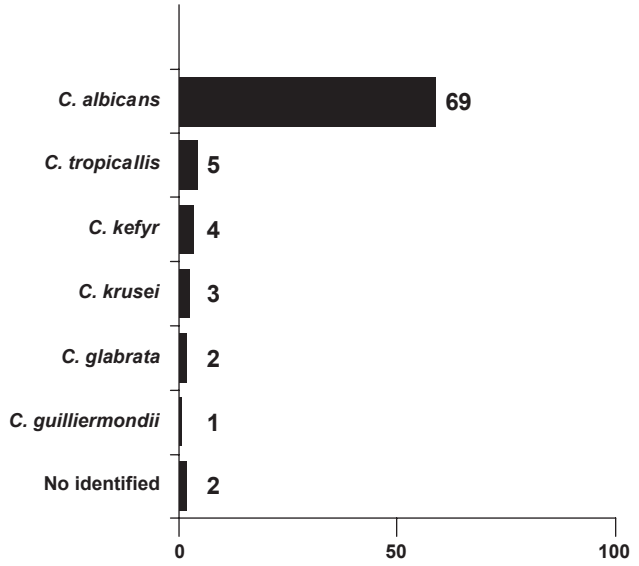

Figure 1 Prevalence of Candida species isolated from oral mucosa of children with AIDS assisted in the ICr/HC - USP, 2006

Severe clinical conditions were prevalent in the sample because 43 patients had severe symptoms of AIDS, 26 presented a high viral load (more than 100 000 HIV-RNA cop ml^{-1}), and almost all of them (109) had already begun antiretroviral therapy at the time of the examination. The positive result for culture was associated with the frequent use of antibiotics (prevalence ratio 1.61; 1.22–2.12) and sulfa drugs (PR = 1.57; 1.20–2.07). A negative association was observed with the use of antiretroviral medicines (PR = 0.63; 0.476–0.85) (Table 2). Two children died during the study period.

The multivariate assessment of covariates fitted a model comprising sociodemographic (household ownership), dental status (dental treatment need), and continuous use of medicines (antiretroviral medicines and antibiotics), which were significantly associated with the positive culture. The selected model explained 7% of the variation in the outcome and correctly identified 63% of the children with AIDS who presented a positive culture (Table 3).

Discussion

A deep immunosuppression, resulting from HIV infection, makes the host more susceptible to the development of some opportunistic infections. Lesions manifested by these infections occur in different ways in different populations, depending on access to medical and dental care, medicine, and nutrition (Pongsiriwet *et al*, 2003; Naidoo and Chikte, 2004). The most common opportunistic infection is oral candidosis, there being only seven cases with oral candidosis among the patients of the present study.

The prevalence of oral colonization by *Candida* yeasts observed in this study was 62% (54–72%, a 95%

Table 1 Assessment of association between positive culture for *Candida sp* in children with AIDS and covariates on socio-demographic and behavioral characteristics. Child's Institute, School of Medicine, University of São Paulo, 2006

Covariates	*Categories*	*Negative Culture*	*Positive Culture*	*Prevalence ratio*[a] *Significance*
Socio-demographic characteristics				
Sex	female	29	34	1.16 (0.85–1.58)
	male	20	34	$P = 0.361$
Skin color:	blacks	31	42	0.96 (0.70–1.32)
	whites	18	26	$P = 0.810$
Schooling caregiver	<11 years	34	54	0.78 (0.51–1.18)
	≥11 years	15	14	$P = 0.234$
Family income	<2 Br. MW[b]	26	39	0.92 (0.67–1.26)
	≥2 Br. MW[b]	23	29	$P = 0.605$
Living conditions				
Household ownership	yes	36	32	0.63 (0.46–0.86)
	no	13	36	$P = 0.003$
Siblings	2 or more	10	26	1.42 (1.06–1.90)
	0 or 1	39	42	$P = 0.019$
Household crowding	≥1 inhab per room	17	25	1.05 (0.76–1.45)
	<1 inhab per room	32	43	$P = 0.757$
Dental status and oral hygiene				
Lifetime dental visits	≥4 times	26	42	1.14 (0.81–1.62)
	<4 times	23	26	$P = 0.454$
Daily tooth brushing	≥3 times	24	24	0.79 (0,5.6–1.10)
	<3 times	25	44	$P = 0.159$
Daily flossing	yes	16	12	0.68 (0.,42–1.08)
	no	33	56	$P = 0.100$
Untreated dental caries	yes	21	41	1.86 (1.28–2.69)
	no	28	27	$P = 0.001$

[a]Adjusted by age.
[b]Brazilian minimum wage, broadly corresponding to us$150.

Table 2 Assessment of association between positive culture for *Candida sp* in children with AIDS and covariates on clinical status and continuous use of medicines. Child's Institute, School of Medicine, University of São Paulo, 2006

Clinical status		*Negative Culture*	*Positive Culture*	*Prevalence ratio*[a] *Significance*
Symptoms	Severe	14	29	1.29 (0.95–1.74)
	Absent, mild or moderate	35	39	$P = 0.103$
Viral load[b]	>100 000	08	18	1.27 (0.93–1.73)
	<100 000	41	50	$P = 0.136$
CD4+ count[c]	Low-for-age	27	34	1.08 (0.79–1.47)
	Normal for age	22	34	$P = 0.624$
Continuous use medicines				
Antiretroviral medicines				
Nucleoside analog reverse transcriptase inhibitors	Yes	48	60	0.63 (0.46–0.85)
	No	01	08	$P = 0.002$
Non-nucleoside analog reverse transcriptase inhibitors	Yes	10	20	1.22 (0.89–1.68)
	No	39	48	$P = 0.213$
Protease inhibitors	Yes	23	28	0.91 (0.66–1.24)
	No	26	40	$P = 0.547$
Antimicrobial medicines				
Sulfametoxazol + trimetropina	Yes	2	12	1.57 (1.20–2.07)
	No	47	56	$P = 0.001$
Antibiotics	Yes	3	17	1.61 (1.22–2.12)
	No	46	51	$P = 0.001$

[a]Adjusted by age.
[b]HIV RNA copies per milliliter of plasma.
[c]CD4 + T-lymphocyte cells per cubic millimeter of blood.

confidence interval), mostly seen among children using antimicrobial medicines, and was lower among those who made use of highly active anti-retroviral therapy (HAART) of nucleoside reverse transcriptase inhibitor (NRTIs) group. It was lowest among those who received HAART associated (NRTIs +nNRTIs or PI+NRTIs) or only non-nucleoside reverse transcriptase inhibitors (nNRTIs) or protease inhibitor (PI). These results differ from the findings of Flanagan *et al* (2000), which showed no statistically significant difference in *Candida* colonization among groups of children subjected to different treatment regimens (dual or triple therapy associated with the protease inhibitor).

Cerqueira *et al* (2010) reported the prevalence of *Candida* spp. in the oral cavities of HIV-infected children compared with those of their seronegative siblings. The authors noted that 80% of HIV-infected children (most of them not being treated by HAART)

Table 3 Multivariate assessment of association between positive culture for *Candida sp* and covariates on sociodemographic, behavioral and clinical characteristics of children with AIDS. Child's Institute, School of Medicine, University of São Paulo, 2006

Sociodemographic characteristics	*Prevalence Ratio* (95% CI)*	P-*value*
Household ownership	0.63 (0.48–0.83)	0.001
Dental status		
Untreated dental caries	1.93 (1.36–2.73)	< 0.001
Continuous use medicines		
NRTIs[a]	0.45 (0.33–0.62)	< 0.001
sulfametoxazol + trimetropina[b]	1.23 (0.97–1.57)	0.085
antibiotics:	1.44 (1.08–1.94)	0.015
Goodness of fit		
-2loglikelihood	195.42	
Pseudo-R2	0.38	
Sensitivity	63%	
Specificity	78%	

*Adjusted by age, and by all covariates included in the model.
[a]NRTIs = nucleoside analogue reverse transcriptase inhibitors
[b]sulfametoxazol + trimetropina: Bactrim®.

had positive culture compared with 60% in seronegative siblings ($P = 0.03$). In both groups, *C. albicans* was the species most frequently found, with a higher prevalence observed in HIV-infected children ($P < 0.05$). The author concluded that HIV-infected children were significantly having more chances of getting colonized by *Candida* spp. The absence of antiretroviral therapy and HAART and carious lesions in the dentin may have influenced the isolation of *Candida* spp. According to the results of the present study, the anti-retroviral therapy acted as a protective factor for oral colonization by *Candida*, but the presence of caries was positively associated with oral colonization by *Candida*.

A strong association was found between *Candida* colonization and the need for dental treatment, with a positive culture in 86% of the cases. This result is in accordance with the findings of the study carried out by Jacob *et al* (1998), in which the authors showed a positive relationship between colonization by *Candida* and carious lesions in the dentin of patients with HIV+/AIDS.

Although *C. albicans* is the species most frequently isolated from the oral mucosa, there is an increased incidence of oral infections by non-*albicans* species (Sánchez-Vargas *et al*, 2005). Among children of ICR/HC, *C. albicans* was the most isolated (80% of cases), whereas the non-*albicans* species accounted for 18% of cases of colonization of the oral mucosa. The second most prevalent species among children of ICR/HC was *C. tropicalis*, with 6% of the isolates; this was much lower than expected when compared with the 55% recorded by Lorena (2000) for a group of HIV-infected children.

Candida dubliniensis is phenotypically similar to *C. albicans*, a fact that has generated discussions on the identification of clinical isolates (Giammanco *et al*, 2002). Several methods for discrimination and identification of *C. dubliniensis* have been reported, including those used in this study, that is, culture medium CHROMagar Candida, and the evaluation of the ability to grow in ASD medium at 45°C (Pinjon *et al*, 1998; Kurzai *et al*, 2000; Giammanco *et al*, 2002). Using these two tests, any presence of *C. dubliniensis* was isolated among the children in this study.

The frequency of oral manifestations in Brazilian and American pediatric AIDS patients has been reported to be influenced by socioeconomic status, behavior, and compliance to treatment (Grando *et al*, 2003). Several studies have focused on the correlation between prevalence of oral lesions and laboratory parameters such as a low CD4 cell count or a high viral load (Ranganathan *et al*, 2000; Aguirre *et al*, 2004). HIV-infected children also suffer from the burden of a higher prevalence of caries compared with their non-infected counterparts (Madigan *et al*, 1996; Castro *et al*, 2001). Among the children in this study, a positive association was found between *Candida* colonization and sociodemographic characteristics; a higher prevalence of colonization by *Candida* was seen in children who did not live in their own homes and who had more than two brothers. These associations suggest that patients with poor socioeconomic conditions are more vulnerable to infection by *Candida*.

Poor socioeconomic status, immunologic alteration, reduced salivary flow, and recurrent use of syrup and sugared pediatric powder for oral solution are usual characteristics of children infected with AIDS (Bretz *et al*, 2000). Such conditions are somehow related to an increased risk of oral manifestations, dental caries in particular.

This study showed a low prevalence rate of oral lesions related to Candida infection, a result compatible with the hypothesis that antiretroviral medicines may have contributed to the reduction of Candida-related oral manifestations. This study also reported a high prevalence of *Candida* colonization in HIV+/AIDS children. This observation reinforces the importance of oral health care in the interdisciplinary health units assisting these patients. Acknowledging the factors associated with *Candida* colonization may help in the development of clinical protocols for monitoring oral health of pediatric patients affected by AIDS.

References

Aguirre JM, Echebarria MA, Eguia-Del Valle A (2004). Síndrome de immunodeficiencia adquirida: manifestaciones en la cavidad bucal. *Med Oral Patol Oral Cir Bucal* **9:** 148–157.

Ahmad S, Khan Z, Mokaddas E, Khan ZA (2004). Isolation and molecular identification of Candida dubliniensis from non-human immunodeficiency virus-infected patients in Kuwait. *J Med Microbiol* **53:** 633–637.

Al-Sweih N, Ahmad S, Khan ZU, Khan S, Chandy R (2005). Prevalence of Candida dubliniensis among germ tubepositive Candida isolates in a maternity hospital in Kuwait. *Mycoses* **48:** 347–351.

Alves SH, Milan EP, de Laet Sant'Ana P, Oliveira LO, Santurio JM, Colombo AL (2002). Hypertonic sabouraud broth as a simple and powerful test for *Candida* dubliniensis screening. *Diagn Microbiol Infect Dis* **43:** 85–86.

Arikan S, Darka O, Hascelik G, Gunalp A (2003). Identification of *Candida dubliniensis* strains using heat tolerance tests, morphological characteristics and molecular methods. *Mikrobiyol Bul* **37:** 49–57.

Barros AJD, Hirakata VN (2003). lternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol* **3:** 21.

Bretz WA, Flaitz C, Moretti A, Corby P, Schneider LG, Nichols CM (2000). Medication usage and dental caries outcome-related variables in HIV/AIDS patients. *AIDS Patient Care STDS* **14:** 549–554.

Brown DM, Jabra-Rizk MA, Falkler WA Jr, Baqui AA, Meiller TF (2000). Identification of *Candida dubliniensis* in a study of HIV-seropositive pediatric dental patients. *Pediatr Dent* **22:** 234–238.

Castro GF, Souza IPR, Oliveira RHS, Portela MB, Esteves C (2001). Prevalência de cárie e sua correlação com a classificação clínica e imunológica em crianças infectadas pelo HIV. *Pesqui Odontol Bras* **15:** 91–97.

Cauda R, Facconelli E, Tumbarello M (1999). Role of protease inhibitors in prevention recurrent oral candidiasis in patients with HIV infection: a prospective case-control study. *J Acquir Immunodefic Syndr* **21:** 20–25.

Centers for Disease Control Prevention (1994). Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR* **43**(RR-12)**:**1–10.

Cerqueira DF, Portela MB, Pomarico L, Soares RMA, Souza IPR, Castro GF (2010). Oral *Candida* colonization and its relation with predisposing factors in HIV-infected children and their uninfected siblings in Brazil: the era of highly active antiretroviral therapy. *Oral Pathol Med* **39:** 188–194.

Cimolai N, Davis J, Trombley C (2002). *Candida* dubliniensis fungemia and vascular access infection. *J Pediatr Hematol Oncol* **24:** 237–239.

European Collaborative Study (1991). Children born to women with HIV-1 infection: natural history and risk of transmission [letter]. *Lancet* **337:** 253.

Flanagan MA, Barasch A, Koenigsberg SR, Fine D, Houpt M (2000). Prevalence of oral soft tissue lesions in HIV-infected minority children treated with highly active antiretroviral therapies. *Pediatr Dent* **22:** 287–291.

Giammanco GM, Pizzo G, Pecorella S, Distefano S, Pecoraro V, Milici ME (2002). Identification of Candida dubliniensis among oral yeast isolates from an Italian population of human immunodeficiency virus-infected (HIV+) subjects. *Oral Microbiol Immunol* **17:** 89–94.

Grando LJ, Yurgel LS, Machado DC *et al* (2003). The association between oral manifestations and the socioeconomic and cultural characteristics of HIV-infected children in Brazil and in the United States. *Pan Am J Publ Health* **14:** 112–118.

Hoare S (2003). HIV infection in children-impact upon ENT doctors. *Int J Pediatr Otorhinolaryngol* **67:** 585–590.

Hoog GS de, Guarro J, Gene J, Figueras MJ (2000). *Atlas of clinical fungi*, 2nd edn. Centraalbureau voor Schimmelcultures: Utrecht.

Jacob LS, Flaitz CM, Nichols CM, Hicks MJ (1998). Role of dentinal carious lesions in the pathogenesis of oral candidosis in HIV infection. *J Am Dent Assoc* **129:** 187–194.

Kozinetz CA, Carter AB, Simon C *et al* (2000). Oral manifestations of pediatric vertical HIV infection. *AIDS Patient Care STDS* **14:** 89–94.

Kurzai O, Korting H-C, Harmsen D *et al* (2000). Molecular and phenotypic identification of the yeast pathogen Candida dubliniensis. *J Mol Med* **78:** 521–529.

Lorena H (2000). *Colonização fúngica da cavidade oral de crianças nascidas de mães soropositivas para HIV no primeiro ano de vida [Dissertação de Mestrado]*. Escola Paulista de Medicina da UNIFESP: São Paulo.

Madigan A, Murray PA, Houpt M, Catalanotto F, Feuerman M (1996). Caries experience and cariogenic markers in HIV-positive children and their siblings. *Pediatr Dent* **18:** 129–136.

Miziara ID, Araujo Filho BC, Weber R (2006). Oral lesions in Brazilian HIV-infected children undergoing HAART. *Int J Pediatr Otorhinolaryngol* **70:** 1089–1096.

Naidoo S, Chikte U (2004). Oro-facial manifestations in paediatric HIV: a comparative study of institutionalized and hospital outpatients. *Oral Dis* **10:** 13–18.

Pincus DH, Coleman DC, Pruitt WR *et al* (1999). Rapid identification of Candida dubliniensis with commercial yeast identification systems. *J Clin Microbiol* **37:** 3533–3539.

Pinjon E, Sullivan D, Salkin I, Shanley D, Coleman D (1998). Simple, inexpensive, reliable method for differentiation of Candida dubliniensis from Candida albicans. *J Clin Microbiol* **36:** 2093–2095.

Pongsiriwet S, Iamaroon A, Kanjanavanti S, Pattanaporn K, Krisanoprakornkit S (2003). Oral lesions and dental caries status in perinatally HIV-infected children in Northern Thailand. *Int J Pediatr Dent* **13:** 180–185.

Ranganathan K, Reddy BV, Kumarasamy N, Solomon S, Viswanathan R, Johnson NW (2000). Oral lesions and conditions associated with human immunodeficiency virus infection in 300 south Indian patients. *Oral Dis* **6:** 152–157.

Sánchez-Vargas LO, Ortiz-López NG, Villar M *et al* (2005). Oral *Candida* isolates colonizing or infecting human immunodeficiency vírus-infected and healthy persons in México. *J Clin Microbiol* **43:** 4159–4162.

Sullivan D, Coleman D (1998). Candida dubliniensis: Characteristics and identification. *J Clin Microbiol* **36:** 329–334.

World Health Organization (1997). *Oral health surveys: basic methods*, 4th edn. World Health Organization: Geneva.

UNAIDS. 2004 report on the global HIV/AIDS edidemic: 4th global report, Available in: http://www.unaids.org/bangkok2004/GAR2004_pdf/UNAIDSGlobalReport2004_en.pdf accessed in 19/11/2009.