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Associations among the use of highly active antiretroviral therapy, oral candidiasis, oral *Candida* species and salivary immunoglobulin A in HIV-infected children

Luciana Pomarico, DDS, MSD [Postgraduate Student]^a, Daniella Ferraz Cerqueira, DDS, MSD [Postgraduate Student]^a, Rosangela Maria de Araujo Soares, PhD [Associate Professor]^b, Ivete Pomarico Ribeiro de Souza, DDS, MSD, PhD [Full Professor]^c, Gloria Fernanda Barbosa de Araujo Castro, DDS, MSD, PhD [Associate Professor]^d, Sigmund Socransky, DDS [Senior Researcher]^e, Anne Haffajee, BDS, DDPH [Senior Researcher]^e, and Ricardo Palmier Teles, DDS, DMSc [Associate Member of the Staff]^f

^aDepartment of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Brazil

^bInstitute of Microbiology Prof. Paulo de Góes, Federal University of Rio de Janeiro, Brazil

^cDepartment of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Brazil

^dDepartment of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Brazil

^eDepartment of Periodontology, The Forsyth Institute, Boston, MA, USA

^fDepartment of Periodontology, The Forsyth Institute, Boston, MA, USA

Abstract

Objectives—To examine the impact of antiretroviral therapy on the prevalence of oral candidiasis, recovery of oral *Candida* species (spp) and salivary levels of total secretory immunoglobulin A (SIgA) and *Candida*-specific SIgA in human immunodeficiency virus (HIV)-infected children.

Methods—Sixty six HIV-positive and 40 HIV-negative children were cross-sectionally examined for the presence of oral lesions. Whole stimulated saliva samples were collected for the identification of *Candida* spp using culture and measurement of total and specific SIgA using enzyme-linked immunosorbent assay (ELISA).

Results—HIV-positive children had a higher prevalence of oral candidiasis ($p < 0.05$); higher frequency of detection of *Candida* spp ($p < 0.05$) and higher levels of total ($p < 0.05$) and *Candida*-specific SIgA ($p < 0.001$) than did HIV-negative children. Among HIV-positive subjects, antiretroviral users had lower viral loads ($p < 0.001$), lower levels of *Candida* spp ($p < 0.05$) and total SIgA ($p < 0.05$) compared with antiretroviral non-users.

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Correspondence to: Luciana Pomarico, Praia do Flamengo, 370/202 – Flamengo, 22210-030 Rio de Janeiro, RJ, Brasil, Fax: 55 (21) 2551-4354, lupomarico@superig.com.br.

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Conclusions—The use of antiretroviral therapy was associated with decreases in the prevalence of oral candidiasis. This diminished exposure to *Candida* spp was accompanied by decreases in levels of total and *Candida*-specific SIgA.

Keywords

HIV infection; child; HAART; *Candida*; secretory IgA

Introduction

Oral candidiasis is the most common opportunistic infection in human immunodeficiency virus (HIV)-positive (HIV+) children and adults and it can cause a considerable level of morbidity.^{1, 2} It has been postulated that co-infection with HIV and *Candida* species (spp) may influence the severity and rate of HIV disease progression in HIV+ individuals.³ Prevalence and severity of oral candidiasis worsen with the progression of the HIV infection and its manifestation is associated with the development of acquired immune deficiency syndrome (AIDS).^{4–6} Several species of *Candida* can colonize the oral cavity and result in candidiasis. Of those, *Candida albicans* is, by far, the most prevalent species in HIV-infected children and adults.^{7, 8} This yeast is also commonly found in healthy individuals from childhood but innate and adaptive salivary immune systems control its growth, averting infection.

Since the introduction of antiretroviral drugs, particularly the highly active antiretroviral therapy (HAART), the prevalence of oral candidiasis in HIV-infected adults^{9–11} and children^{12–16} has decreased dramatically. Two potential mechanisms account for this finding, the immune reconstitution induced by HAART and an antifungal effect.¹⁷ HAART results in an increase in the levels of CD4+ T lymphocytes rescuing the immune system,¹⁸ while the protease inhibitors present in the drug cocktail could interfere with secreted aspartic proteinases (SAPs), the main proteases secreted by *C. albicans*, hampering its proliferation and pathogenicity.¹⁹

Due to the pivotal role of CD4+ T lymphocytes in the maturation of the oral secretory immune system, a failure in this immunity as a consequence of the HIV infection has been proposed.²⁰ Early reports have suggested a decrease in the levels of *C. albicans*-specific secretory immunoglobulin A (SIgA) in the oral cavity of HIV-positive adults having oral candidiasis, compared with those without it.²¹ However, other studies have reported an increase in the levels of anti-*C. albicans* SIgA in HIV-infected subjects compared with HIV-negative (HIV–) individuals.^{22–24} Positive associations between increased levels of anti-*C. albicans* SIgA and the presence of oral candidiasis have also been described.²³ These findings suggest that the salivary secretory immune system maintains its responsiveness despite HIV infection. However, studies comparing the levels of salivary IgA in HIV-infected subjects with and without AIDS have indicated that in the more advanced stages of HIV-induced immunosuppression, the oral secretory immune system might become less effective.^{25, 26}

Although several studies have examined the presence of oral candidiasis in HIV-infected children and suggested a decrease in the prevalence of these lesions after the introduction of HAART, there are no studies in the literature examining, at the same time, correlations among the use of antiretroviral therapy and HAART and the salivary levels of *Candida* spp and SIgA. Therefore, the goal of the present study was to examine the impact of the use of antiretroviral therapy, particularly HAART, on the prevalence of oral candidiasis, recovery of *Candida* spp from the oral cavity and salivary levels of total SIgA and *Candida*-specific SIgA in children infected with HIV. In addition, we explored associations among these clinical, microbiological and immunological parameters with the degree of immunosuppression of the study subjects.

Methods

Study population

The subjects of this cross-sectional study consisted of 65 children infected with HIV by vertical transmission (HIV-positive group) and 40 HIV-seronegative children, who were siblings of HIV-positive children (HIV-negative group). Enrolled children were 2 to 14 years of age, and the groups were gender and age matched. All HIV-positive patients attended the Pediatric AIDS Outpatients Clinic at the Federal University of Rio de Janeiro, Brazil, and had definitive diagnosis for HIV infection confirmed by two positive enzyme-linked immunosorbent assay (ELISA) tests and one positive Western Blot. The seronegative status of the control group also was confirmed by ELISA and Western Blot. Exclusion criteria included systemic or local treatment with antifungals or antibiotics within the previous three months. The study was approved by the Ethics Committee of the Federal University of Rio de Janeiro, and informed consent forms were obtained from all the children's legal guardians.

Sample collection and clinical examination

Sample collection for microbiological and immunological analyses and the clinical examination were performed by two calibrated pediatric dentists. Subjects were instructed not to eat, drink or brush their teeth for a period of two hours prior to their sampling appointment. After chewing on a paraffin stick (1 gram), subjects expectorated whole stimulated saliva into sterile vials for five minutes until approximately 500 µl of saliva had been collected. Suction bulbs (Sigma®, Brazil) were used whenever children were unable to expectorate. Since the study population included very young children, salivary flow could not be determined reliably, as several children swallowed saliva during sample collection. All samples were refrigerated and transferred to a laboratory within two hours after collection. Saliva samples were cleared by centrifugation at 9,300 g for 10 min to pellet bacteria and the supernatant was kept at -80° C until assayed. Following sample collection, all children were assessed for oral lesions. The following data were collected from the children's medical records: personal information, medical history and results from laboratory tests (the closest ones to the time of sample collection) including immunological and clinical classification (CDC classification²⁷), percentage of CD4- and CD8-positive cells, CD4/CD8 ratio, viral load, diagnosis of AIDS, antiretroviral therapy, HAART and use of antifungal or other medication. Antiretroviral therapy involved the use of one or more nucleoside reverse transcriptase inhibitors (NRTI) with or without non-nucleoside reverse transcriptase inhibitors (nNRTI). Patients were considered to be using HAART whenever protease inhibitors (PIs) were added to their antiretroviral regimen.

Microbiological analysis

Each saliva sample was diluted 1:10 with 0.9% sterile saline solution (pH 7.2) without bacteriostatic agents. Aliquots of 100 µl were cultured on plates with a chromogenic agar (CHROMagar *Candida*®) and incubated at 37°C for 48–72 hours. The culture medium allows the presumptive identification of *Candida* spp based on the color of each colony.²⁸ Colonies were counted on plates with positive growth to calculate the number of colony forming units (CFU) per ml of saliva. The positive growths were classified according to Lamey et al.²⁹ as mild (< 10 CFU/mL), moderate (11–49 CFU/mL) or strong (> 50 CFU/mL) growth. Plates exhibiting no growth were incubated for another 24 hours to confirm the absence of *Candida* spp colonies.

Green colonies were inoculated onto Sabouraud dextrose agar to screen for the ability to grow at 45°C, for 48 hours, to differentiate *C. dubliniensis* and *C. dubliniensis*, since the latter fails to grow at 45°C.³⁰ In addition, each different colored colony was characterized by sugar substrate assimilation profiles using the API 20C system® (bioMérieux, France), which allows

the phenotypical differentiation of clinical isolates according to their sugar assimilation and fermentation reactions. The sugars methyl- α D-glucopyranoside and D-xylose (API 20D system) were also tested to differentiate *C. dubliniensis* and *C. dubliniensis*.

Immunological analysis

The salivary concentration of total SIgA was determined using a commercial indirect competitive ELISA kit (Salimetrics LLC, State College, PA), specifically designed for saliva samples. In brief, goat anti-human SIgA conjugated to horseradish peroxidase (HRP) was added to standards (ranging from 2.5 to 600 μ g/ml) and saliva samples (diluted 5-fold) and incubated at room temperature for 90 min. After incubation, standards and saliva samples were added to a microtitre plate coated with human SIgA and incubated at room temperature for 90 min on a rotator (400 rpm). After incubation and wash of unbound components, captured conjugate was measured by the addition of the substrate tetramethylbenzidine. Optical density was read at 450 nm on a plate reader (BIO-TEK Instruments. Inc., Winooski. VT). The results from the standard curve were plotted using a semi-logarithmic scale, and a curve generated. The concentrations of total SIgA were determined by comparing the optical density data to a standard curve using a four parameter logistic-log model and the ELISA for Windows software (Centers for Disease Control and Prevention, Atlanta, GA).

Specific SIgA for *C. albicans* was measured using a modified ELISA. Microplates (Costar) were coated with formalin-killed *C. albicans* (ATCC 10231) in PBS at 3×10^7 cells/ml, and incubated for three hours at room temperature and transferred to a cold room. After at least two days of incubation at 4°C, the plates were washed and incubated with diluted saliva samples (1:5 in blocking buffer [0.1% bovine albumin in PBS-Tween]) overnight at 4°C. Captured SIgA was detected by incubation with monoclonal mouse anti human secretory component diluted in blocking buffer (1:1,000) (Sigma. St. Louis. MO). Bound primary antibody was detected by reaction with biotinylated goat anti-mouse IgG diluted in blocking buffer (1:20,000) (Sigma. St. Louis. MO) followed by incubation with streptavidin-HRP conjugate diluted in blocking buffer (1:500). Substrate (Substrate Reagent Pack, R&D Systems) was added to each well and after 30 minutes the reaction was stopped by the addition of 1N NaOH. Conversion of substrate was determined at 405 nm using an ELISA reader (BIO-TEK Instruments. Inc., Winooski. VT.). Salivary antibody concentrations were calculated by reference to a pool of standard saliva samples obtained from subjects with high levels of antibody activity, which was assigned the value of 1000 ELISA units (EU). The results were plotted on a semi-logarithmic scale, and a curve generated. The concentrations of *C. albicans*-specific SIgA were determined using linear regression and the software Prism 5 for Windows (GraphPad Software, Inc., San Diego, CA).

Data Analysis

Significance of differences between HIV-negative and HIV-positive children and AIDS-negative (AIDS-) and AIDS-positive (AIDS+) children was determined using the Mann-Whitney test for the variables: age, yeast CFU/ml, total SIgA and *Candida*-specific SIgA. Significance of differences for the percentage of males, prevalence of the various oral lesions and percentage of subjects with *Candida* spp carriage was tested using chi-square test. The Mann-Whitney test was also used to examine the significance of differences between AIDS-negative and AIDS-positive subjects regarding the percentage of CD4+ and CD8+ T cells, the CD4/CD8 ratio and their viral load. Relationships among clinical, microbiological and immunological variables were explored using the Phi correlation coefficient for correlations between dichotomous data; the Biserial correlation coefficient for correlations between dichotomous and quantitative data and the Spearman correlation coefficient for correlations between continuous data.

In order to examine the effects of antiretroviral therapy and HAART on the clinical, microbiological and immunological parameters, the HIV-positive subjects were separated into three groups: 1) antiretroviral non-users (AR-), 2) antiretroviral users (AR+) and 3) HAART users (HAART+). Groups were compared using the Kruskal-Wallis test and the chi-square test, depending on the nature of the variable.

Results

Subject Population and Clinical Manifestations

The study population was composed of 105 subjects, 65 HIV-positive children and 40 HIV-negative siblings of HIV-positive children varying in age from 2 to 14 years. The two groups had similar age and gender distribution (Table I). In HIV-negative children, gingivitis was the only oral lesion detected. Oral candidiasis and recurrent oral candidiasis were detected significantly more frequently in HIV-positive children. Other oral lesions also found in HIV-positive children were: cheilitis, linear gingival erythema (LGE), labial herpes and ulcers, but differences from HIV-negative children were too few in number to be significant. The prevalence of gingivitis was similar in the two groups (Table I).

Sixty three percent of the HIV-infected children had AIDS. Seventy six percent of the HIV-positive children were on antiretroviral therapy but only 36% were taking HAART. Table II presents the clinical and immunological data for the HIV-positive children divided into AIDS-negative (n = 24) and AIDS-positive (n = 41) subjects. AIDS-positive children had a significantly lower mean % of CD4+ cells and a lower mean CD4/CD8 ratio. There was no difference in the viral load ($\times 10^4$) between AIDS- negative and AIDS-positive children. The prevalence of several oral lesions (i.e., oral candidiasis, recurrent oral candidiasis, gingivitis, herpes simplex virus infection) was higher in AIDS individuals than in AIDS-negative children but differences were not statistically significantly. Cheilitis, linear gingival erythema (LGE) and ulcers were only identified in AIDS patients.

Oral Candida spp Isolation

HIV-positive children presented a significantly higher percentage of subjects with positive growth of yeast than HIV-negative children (80% vs. 57%, respectively; $p < 0.05$). However, HIV-negative subjects presented a significantly higher mean yeast concentration in saliva (77.1 ± 230.4 CFU/mL) than HIV-positive subjects (68.5 ± 178.2 CFU/mL). When the median was compared between the two groups, the results were 2.0 ± 230.4 and 18.5 ± 178.2 for HIV-negative and HIV-positive subjects, respectively. Of the *Candida* spp identified, *C. albicans* was the most prevalent in HIV-negative children (74% [of the yeast positive subjects]), followed by *C. parapsilosis* (43%), *C. guilliermondii* (17%), *C. tropicalis* (17%), *C. krusei* (9%), *C. dubliniensis* (4%) and *C. glabrata* (4%). In HIV-positive subjects the frequencies of detection of the different *Candida* spp were: *C. albicans* (94%), *C. parapsilosis* (6%), *C. guilliermondii* (23%), *C. tropicalis* (17%), *C. krusei* (2%), *C. dubliniensis* (2%), *C. glabrata* (2%) and *C. lusitaniae* (8%). When the two groups were compared using the chi-square test, *C. albicans* was significantly more prevalent in HIV-positive than in HIV-negative children ($p < 0.05$), while *C. parapsilosis* was significantly more prevalent in HIV-negative children compared to HIV-positive subjects ($p < 0.001$).

Total secretory IgA and SIgA specific for Candida

HIV-positive children had significantly higher levels of total SIgA $\mu\text{g/ml}$ (141.0 ± 75.2 [mean $\pm$ SD]) than their HIV-negative siblings (111.0 ± 59.1) ($p < 0.05$) (Table I). Levels of SIgA specific for *Candida* measured in ELISA units (EU) were also significantly elevated in the HIV-positive children (39.6 ± 67.5) compared to HIV-negative siblings (16.6 ± 8.7) (Table I). In the HIV-positive group, AIDS-positive subjects had significantly lower levels of total SIgA

(126.7 ± 69.0) compared to AIDS-negative subjects (165.5 ± 80.3) ($p < 0.05$), while the levels of anti-*Candida* SIgA were higher in AIDS-positive (44.3 ± 83.4) children compared to AIDS-negative children (31.5 ± 21.3), but differences were not statistically significant (Table II).

Correlations among clinical, immunological and microbiological parameters

A significant positive correlation was found between *Candida*-specific SIgA and yeast CFU/mL of saliva (Spearman $r = 0.26$, $p < 0.01$) as well as a significant correlation between total SIgA and *Candida*-specific SIgA (Spearman $r = 0.27$, $p < 0.05$), using data from all subjects. Within the HIV+ group, several significant negative associations were found among clinical parameters and immunological parameters including the diagnosis of AIDS, recurrent oral candidiasis and gingivitis with %CD4+ cells (Table III). The levels of total SIgA also had a significant negative association with the presence of AIDS. The use of antiretrovirals was inversely associated with the viral load, the detection of yeast in saliva and the presence of oral candidiasis, while the use of HAART also had a negative correlation with the isolation of *Candida* spp in saliva.

Effects of antiretroviral therapy and HAART on clinical, immunological and microbiological parameters

All clinical, immunological and microbiological parameters were compared among antiretroviral non-users (AR-), antiretroviral users (AR+) and HAART users (HAART+) groups. The results reported in Table IV demonstrate that the prevalence of AIDS was significantly higher among HAART users, followed by patients on antiretroviral drugs and patients in the antiretroviral non-users group. Differences among groups were also significant for viral load, percentage of subjects with positive growth of *Candida* spp, yeast CFU/mL and total SIgA, with values for AR- > AR+ > HAART+ subjects. A similar trend was found for *Candida*-specific SIgA, but differences were not significant. The prevalence of oral candidiasis was highest in the non-medicated group, but differences were not significant. Conversely, recurrent oral candidiasis was diagnosed more frequently in HAART users, followed by AR+ and AR- subjects.

Discussion

Our data have confirmed the responsiveness of the SIgA system to candidal infections in HIV positive children even with clinical progression to AIDS. The high titers of *Candida*-specific SIgA in HIV-positive children resulted from a higher prevalence of oral candidiasis and colonization by *Candida* spp. Antiretroviral therapy and HAART were accompanied by immune reconstitution and a decrease in prevalence of oral candidiasis and *Candida* spp carriage. The decrease in antigenic exposure resulted in a decrease in levels of *Candida*-specific SIgA. The addition of protease inhibitors to the antiretroviral regimen in HAART resulted in further decreases in oral candidiasis prevalence and candidal carriage, which might have resulted from the direct anti-yeast effects of the protease inhibitors. To our knowledge, this is the first report to examine simultaneously the impact of antiretroviral therapy on the clinical manifestation of candidiasis and on microbiological and immunological parameters associated with this infection.

In accord with previous reports,^{12–15} HIV-infected children had a higher prevalence of oral lesions, particularly current and recurrent oral candidiasis, compared with non-infected children. Within the HIV-infected group, when the most immunocompromised children with the diagnosis of AIDS were singled out, these subjects presented significantly higher prevalence of several oral lesions when compared with AIDS-infected individuals, including: oral candidiasis, recurrent oral candidiasis, gingivitis and labial herpes (Table II). A Biserial correlation coefficient of -0.34 between the percentage of CD4+ cells and the prevalence of

recurrent oral candidiasis ($p < 0.01$) confirmed the association between the level of immunosuppression, and the occurrence of oral candidiasis. Our data on the isolation of *Candida* spp from the oral cavity of HIV-positive and HIV-negative children paralleled the clinical diagnosis of candidiasis. The prevalence of yeast carriage in the HIV-positive group was significantly higher than the HIV-negative group; however, the mean yeast CFU/mL of saliva was higher in the HIV-negative group. This finding could be explained by the highly skewed distribution of values in the HIV-negative children, with outlier values inflating the mean. In contrast, HIV-negative subjects had lower median yeast concentrations (2.0 vs. 18.5) CFU/mL than the HIV-positive subjects.

C. albicans was the most prevalent *Candida* spp and it was more prevalent in HIV+ subjects compared to HIV- individuals, while *C. parapsilosis* was significantly more frequently detected in HIV- children. In accordance with these findings, Contreras et al.³¹ observed that *C. parapsilosis* was the most abundant species in the oral cavity of healthy children. However, over time, this *Candida* spp was gradually replaced by *C. albicans*, possibly due to its greater pathogenic potential and antifungal resistance.

In the present study, the control group was composed of HIV-negative siblings of HIV-positive children. This mode of selecting control subjects, used previously by Madigan et al.,³² presents several advantages over randomly selected control subjects: 1) the need to test for HIV seroconversion is obviated since siblings of HIV-positive subjects are constantly tested for HIV infection; 2) it compensates for several environmental variables that might impact the prevalence of oral candidiasis and *Candida* spp carriage, since subjects are living within the same household; 3) it provides an even distribution across socioeconomic strata of case and control groups and 4) it results in a homogeneous *Candida* spp challenge via vertical and horizontal transmission.

Assessments of oral mucosal secretory function through measurements of total SIgA and *Candida*-specific SIgA, confirmed previous reports suggesting the maintenance of the responsiveness of the SIgA system during HIV infection.^{22, 23, 33–35} Both total and specific IgA were elevated in HIV+ children, possibly as a response to the elevated stimulus provided by the opportunistic infections that accompany the progression of the HIV infection. We found a significant correlation between the yeast CFU/mL of saliva and the salivary levels of *Candida*-specific SIgA, suggesting that the secretory immune system was responding directly to the challenge presented by the *Candida* colonization or infection. Similar findings were described by Coogan et al.,²² who reported a significant correlation between whole saliva IgA antibodies to *Candida* spp with the numbers of *Candida* organisms isolated from saliva. In AIDS + patients there was a significant decrease in total SIgA, compared to AIDS- children. In addition, the presence of AIDS had a significant negative correlation with the salivary levels of SIgA. The implication of these findings is that with advanced immunosuppression, the secretory immune system starts to show signs of decay. Other reports have also described lower mean antibody levels in AIDS patients compared with HIV+ subjects.^{22, 25} However, the specific secretory immune response to *Candida* remained elevated despite the advent of AIDS, and the higher colonization by yeast in AIDS subjects was followed by a higher level of *Candida*-specific immunoglobulins.

HAART decreases the prevalence of oral lesions; particularly oral candidiasis in HIV-infected children.^{13, 36} Two mechanisms have been proposed to explain this finding: 1) a reduction in the infection as a result of the immune reconstitution resultant from the increase in numbers of CD4+ cells, and 2) a potential direct anti-yeast effect.¹⁸ The protease inhibitors present in HAART can also inhibit candidal SAPs, interfering with the growth and pathogenicity of *Candida* spp.^{19, 37} In a previous report, the start of HAART resulted in significant decreases in episodes of oral candidiasis, *Candida* carriage and salivary SAPs only when protease

inhibitors were part of the cocktail of antiretroviral drugs.³⁸ We also documented the beneficial effects of the use of antiretrovirals and HAART in several clinical and immunological parameters of the HIV infected children. Antiretroviral therapy was associated with significantly lower viral load, yeast CFU/mL and detection of *Candida*. The guidelines followed in Brazil for antiretroviral therapy of children recommend the administration of combination therapy for patients who are symptomatic or have moderate to severe immunosuppression. Therefore, it was not surprising to observe that the prevalence of AIDS was significantly higher among HAART users, followed by antiretroviral users and antiretroviral non-users children.

Despite the higher number of AIDS cases in the HAART+ and AR+ groups compared with the AR- group, there were no differences in the levels of CD4+ cells among the 3 groups, suggesting that the antiretroviral therapies resulted in immune reconstitution. HAART+ and AR+ subjects also had a lower prevalence of oral candidiasis than AR- individuals and lower levels of total SIgA and *Candida*-specific SIgA. These associations suggest that either direct or indirect anticandidal effects of the antiretroviral therapies could be occurring, resulting in less yeast carriage and infection, inducing a decrease in the SIgA response.

The primary difference between antiretroviral users and HAART users was the use of protease inhibitors in the HAART regimen. This allowed us to make inferences about the potential direct anticandidal effects of protease inhibitors. The data suggested that the addition of protease inhibitors to the patient's drug regimen was associated with lower *Candida* carriage and infection, supporting an enhanced anticandidal effect. Although it is also possible that HAART resulted in a better immune reconstitution compared to the other antiretroviral regimens and an indirect anti-*Candida* effect, the lack of difference in the percentage of CD4+ cells between the two groups argues against this hypothesis. These findings contrast with a recent study showing that prolonged HAART resulted in a major reduction in the prevalence of oral candidiasis from 10.6% to 2.1% ($p < 0.01$), while the impact on *Candida* recovery was less impressive, decreasing in prevalence from 57.0% to 46.5% ($p > 0.05$).³⁷ In our sample the prevalence of oral candidiasis in HAART+ and AR+ children was quite similar; 8% vs. 4%, respectively (Table VI). Conversely, *Candida* spp was found in 58% of HAART users compared to 88% of antiretroviral users patients and 100% of antiretroviral non-users subjects ($p < 0.05$ [Table IV]). There were also significant differences in the mean yeast CFU/mL of saliva between the two groups; 21.3 vs. 83.5 for HAART and antiretroviral therapy users, respectively ($p < 0.05$). Collectively these data suggest an anti-yeast effect of the protease inhibitors in HAART. It must be mentioned that a potential limitation of our study was the fact that we did not collect information on the duration of AR or HAART use.

In summary, our data suggested that the use of antiretroviral therapy in HIV-positive children was associated with immune reconstitution, decreases in the prevalence of oral candidiasis and a lower *Candida* spp carriage. The decrease in exposure to *Candida* spp was accompanied by a decrease in levels of total SIgA and *Candida*-specific SIgA. Children who used protease inhibitors as part of the antiretroviral regimen in HAART showed additional reductions in oral candidiasis prevalence and candidal carriage. It is possible to speculate that the protease inhibitors might have had an anti-candidiasis effect, resulting not only from the immune reconstitution, but also from a direct anti-yeast mechanisms.

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Table I

Characteristics of 40 HIV-negative (HIV-) and 65 HIV-positive (HIV+) children

	HIV-	HIV+	p-value
n	40	65	
% males	45	42	NS*
Age (years)	8.1 ± 3.3	8.5 ± 2.7	NS**
% of subjects with:			
oral candidiasis (OC)	0	11	p < 0.05*
recurrent OC	0	35	p < 0.001*
cheilitis	0	5	NS*
linear gingival erythema	0	2	NS*
gingivitis	35	30	NS*
herpes simplex virus infection	0	6	NS*
ulcers	0	3	NS*
oral <i>Candida</i> carriage	57	80	p < 0.05*
means ± SD			
yeast CFU/mL	77.1 ± 230.4	68.5 ± 178.2	p < 0.05**
Total SIgA (ng/μl)	111.0 ± 59.1	141.0 ± 75.2	p < 0.05**
anti-C. albicans SIgA (EU)	16.6 ± 8.7	39.6 ± 67.5	p < 0.001**

* Tested using the chi-square test

** Tested using the Mann-Whitney test

Table II
Characteristics of HIV-positive children by AIDS status

	AIDS-	AIDS+	p-value
n	24	41	
% ARV users	11 (45.8%)	38 (92.7%)	p < 0.01 *
% HAART users	3 (12.5%)	21 (51.2%)	p < 0.01 *
%CD4+ cells	25	20	p < 0.01 *
%CD8+ cells	48	51	NS **
CD4/CD8	0.55	0.50	p < 0.05 **
viral load ($\times 10^4$)	8.6 $\pm$ 14.5	7.6 $\pm$ 12.0	NS **
% of subjects with:			
oral candidiasis (OC)	4	14	NS *
recurrent OC	21	43	NS *
cheilitis	0	7	NS *
linear gingival erythema	0	2	NS *
gingivitis	21	36	NS *
herpes simple $\times$ virus infection	4	7	NS *
ulcers	0	5	NS *
oral <i>Candida</i> carriage	88	76	NS *
means $\pm$ SD			
yeast CFU/mL	46.9 $\pm$ 87.4	80.9 $\pm$ 213.6	NS **
Total SIgA (ng/ μ l)	165.5 $\pm$ 80.3	126.7 $\pm$ 69.0	p < 0.05 **
anti-C. albicans SIgA (EU)	31.5 $\pm$ 21.3	44.3 $\pm$ 83.4	NS **

* Tested using the chi-square test

** Tested using the Mann-Whitney test

Table III

Significant correlations among clinical, microbiological and immunological variables in HIV-positive children

Variables		Correlation Coefficient	p-value
AIDS	%CD4+	-0.24 [*]	p < 0.05
AIDS	Total SIgA	-0.25 [*]	p < 0.05
gingivitis	%CD4+	-0.29 [*]	p < 0.05
recurrent OC	%CD4+	-0.34 [*]	p < 0.01
recurrent OC	oral candidiasis	-0.37 ^{**}	p < 0.01
antiretroviral	viral load ($\times 10^4$)	-0.30 [*]	p < 0.01
antiretroviral	oral candidosis	-0.26 ^{**}	p < 0.05
antiretroviral	+ve growth	-0.28 ^{**}	p < 0.05
HAART	+ve growth	-0.42 ^{**}	p < 0.001

* Biserial correlation coefficient

** Phi correlation coefficient

Table IV

Characteristics of HIV-positive children by AR and HAART use

	AR-	AR+	HAART+	p-value
n	16	25	24	
% males	31	42	50	NS*
Age (years)	7.1 ± 3.1	8.9 ± 2.4	9.0 ± 2.6	NS**
% AIDS	19	69	88	p < 0.001*
%CD4+ cells	23	21	22	NS**
%CD8+ cells	49	49	51	NS**
CD4/CD8	0.48	0.50	0.57	NS**
viral load (× 10 ⁴)	14.8 ± 18.6	7.1 ± 10.2	4.4 ± 9.0	p < 0.01**
% of subjects with:				
oral candidiasis (OC)	25	4	8	NS*
recurrent OC	25	31	46	NS*
oral <i>Candida</i> carriage	100	88	58	p < 0.01*
means ± SD				
yeast CFU/mL	115.1 ± 254.8	83.5 ± 196.9	21.29 ± 34.4	p < 0.05**
Total SIgA (ng/μl)	188.2 ± 87.2	130.6 ± 64.8	119.9 ± 65.4	p < 0.05**
anti-C. albicans SIgA (EU)	51.2 ± 74.7	42.0 ± 87.5	28.9 ± 22.5	NS**

* Tested using the chi-square test

** Tested using the Mann-Whitney test