Examining Dentinal Carious Lesions as a Predisposing Factor for the Oral Prevalence of *Candida* spp in HIV–infected Children

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ABSTRACT

Purpose: The aims of this study were to verify the oral prevalence of *Candida* spp in HIV-infected children, and investigate the association between *Candida* colonization and dentinal caries lesions.

Methods: Whole stimulated saliva was collected from 62 HIV-infected children (group 1) and 40 seronegative siblings (group 2), followed by oral examination to determine: dmft/dmfs scores, DMFT/DMFS scores, the number of dentinal carious teeth (D+) and the presence of oral candidiasis. The salivary samples were cultured, and plates with positive isolation (G+) were classified as mild growth, moderate growth, and strong growth. Data was analyzed using chi-square, Mann-Whitney, and Spearman tests for correlations.

Results: The patients' mean age was 8.8 for group 1 and 8.0 years for group 2. In group 1, 61% of the subjects had AIDS. Eighty percent of HIV-infected children (N=50) were positive for *Candida* growth, having a mean CD4% of 22, those who were *Candida*-free (N=12) presented a mean CD4% of 21. Correlation was observed between the mean D+ and G+ in groups 1 and 2 ($P<.05$, Mann-Whitney test), but not between the mean dmft/dmfs-DMFT/DMFS in group 1 ($P>.05$, Mann-Whitney test). Association of G+ and the D+ was noted in group 1 ($P<.05$; chi-square test). Positive correlation between high *Candida* counts and an increase in D+ was demonstrated in groups 1 and 2 ($P<.05$).

Conclusions: Dentinal carious lesions may be associated with *Candida* spp colonization in HIV-infected children. (J Dent Child 2007;74:98-103)

KEYWORDS: CARIES, CANDIDA, PEDIATRIC AIDS

Among the possible first signs and symptoms of HIV-infection in children[1-4] are oral manifestations such as oral candidiasis, herpes simplex infection, linear gingival erythema, parotid enlargement, and recurrent aphthous ulcers. Their presence is usually associated with the advance and severity of HIV-infection,[4-5] especially for candidiasis lesions.[4-8]

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Oral candidiasis (OC) is the most common opportunistic infection,[1-11] and may occur in up to 72% of all pediatric HIV-infection cases.[3] It is considered an infection of significant value in predicting the evolution of the AIDS disease in these patients, once they are considered markers of immunossuppression.[1-11]

OC is an infection of fungal etiology mainly caused by *Candida albicans*,[2,3,10,17] although other species are often associated such as *Candida tropicalis*, *Candida stellatoidea*, *Candida krusei*, *Candida parapsilosis*, *Candida glabrata*,[2,17,18] and *Candida dubliniensis*.[17-21] *C albicans* and related species commonly inhabit the human oral cavity, with a prevalence ranging from 3% to 60%.[12,14] In HIV-infected patients, the prevalence rates can vary as well, from 19% to 81%.[15-17] Some predisposing factors that influence the

development of OC include: immunosuppression; endocrinal diseases; xerostomia; poor oral hygiene; antibiotics; and steroid therapy.[22] Many of them may be present in HIV-infected patients.[23] Investigations have shown an association between dentinal carious lesions and oral colonization by *Candida* spp, indicating that these lesions may serve as an oral reservoir for fungal organisms.[24-27] Moreover, Sziegoleit et al[26] and Rego et al[28] observed a reduction of *Candida* spp counts after dental treatment. Additionally, Jacob et al (1998)[25] found that carious dentinal tubules were colonized by *Candida* spp in HIV-infected adults more frequently than seronegative patients.

Therefore, the aims of this study were to: describe the prevalence of *Candida* spp in the oral cavity of HIV-infected children; and investigate the correlation between the presence of dentinal carious lesions and *Candida* spp growth.

METHODS

Subjects of this study consisted of 62 vertically HIV-infected children from 2 to 13 years old who comprised group 1. All were patients of a pediatric AIDS outpatient clinic associated with the Federal University, Rio de Janeiro, Brazil, and were participants of a health care program provided by the pediatric dentistry department of the same university. All children included had definitive diagnosis of HIV infection confirmed by 2 positive ELISA tests and 1 positive Western Blot.

The control group (group 2) included 40 HIV-seronegative children—confirmed by the above cited tests—who were siblings of HIV-positive children and were matched in gender and age. The criteria for exclusion for both groups were the: presence of fixed or removable orthodontics appliances, and systemic or local treatment with antifungal within the last 3 months, and use of antimicrobials for group 2.

This study was approved by the ethics committee of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, and informed consents were obtained from all the children's parents/legal guardians.

Sample collection for microbiological investigation was performed by a single trained pediatric dentist. The whole stimulated saliva samples were obtained by asking the patient to chew a paraffin stick (1 g) and expectorate of at least 2 ml of saliva in a sterilized container. Suction bulbs (Sigma, Brazil) were used when children were unable to expectorate the saliva.[29] Salivation rates were not calculated. All samples were kept refrigerated for laboratorial procedures within 2 hours.

Afterwards, all children underwent supervised toothbrushing with fluoridated toothpaste followed by topical fluoride application (2.0% sodium fluoride). Plaque scores, however, were not recorded. Oral examination was then performed for assessment of orofacial lesions, DMFT/DMFS and dmft/dmfs scores and the number of teeth with dentinal carious lesions of each child (only open cavities were recorded), based on visual and tactile (with an OMS probe) examination. Patients with dental needs were referred to the dental clinic of the same university. They also received oral hygiene and dietary instructions for maintenance of the oral health.

Collected from their medical records were all data regarding the patients' personal information and medical history (use of antifungal or other medications; Centers for Disease Control and Prevention classification[30] [1994 Revised classification system for HIV infection in children less than 13 years of age; N=no symptoms; A=mild symptoms; B=moderate symptoms; C=severe symptoms (AIDS); 1=absence of immunosuppression (CD4%>25); 2=moderate immunosuppression (CD4%=15-24); 3=severe immunosuppression (CD4%<15; AIDS)].

LABORATORIAL ANALYSIS

The saliva was diluted at a 1:10 ratio with 0.9% sterile saline solution (pH 7.2) without bacteriostatic agents. Aliquots of 100µl were cultured in plates with a chromogenic agar (CHROMagar Candida®) incubated at 37°C for 48 to 72 hours, which allows a presumptive identification of *Candida* spp through the color of each colony formed.[30] Plates with positive growth were classified according to Lamey et al (1988)[31] as: (1) mild growth (M; <10 cfu/ml); (2) moderate (MM; 11-49 cfu/ml); and (3) heavy (H; >50 cfu/ml). Those without growth were left incubated for another 24 hours to confirm the absence of *Candida* spp colonies.

Laboratorial procedures were performed by the same pediatric dentist, who collected dental examination data and was trained in microbiology. All microbiology analysis was evaluated blindly regarding the patients' medical history.

Data was analyzed descriptively via a statistical program (SPSS v. 11.0, SPSS Inc, Chicago, Ill) using the chi-square, Mann-Whitney, and Spearman's correlation tests with a 5% level of significance.

RESULTS

The group 1 subjects' mean age was 8.8 years (±2.7), as represented by 42% of group 1 boys The group 2 patients' mean age was 8.0 years (±3.2), as represented by 55% of

Table 1: Medical data from HIV-infected children (n=62).

Immunological Classification*	*Clinical Classification	AIDS	Antiretroviral Therapy	HAART
1 23 (37.1%)	A 12 (19.4%)	Yes- 38 (61.3%)	Yes- 46 (74.2%)	Yes- 23 (37.1%)
2 18 (29.0%)	B 16 (25.8%)	No- 24 (38.7%)	No- 16 (25.8%)	No- 39 (62.9)
3 21 (33.9%)	C 32 (51.6%)			
	N 02 (03.2%)			

** 1994 Revised classification system for Human Immunodeficiency Virus infection in children less than 13 years of age (CDC). 1–absence of immunessupression (CD4 % > 25); 2– moderate immunessupression (CD4 % =15–24); 3– severe immunessupression (CD4% <15) (AIDS) N– no symptoms; A– mild symptoms; B–moderate symptoms; C–severe symptoms (AIDS)23.*

group 2 girls. Considering the CDC[30] classification, a large proportion of group 1 patients (N=38/62, 61%) was classified as having AIDS and moderate to severe immunosuppression (N=32/62, 52%). Medical data from group 1 patients are described in Table 1.

Considering growth for *Candida* spp, 80% of the sample (N=50) was positive, with: (a) 30% (N=15) presenting mild growth, (b) 34% (N=17) presenting moderate growth; and (c) 36% (N=18) representing heavy growth. The subjects with positive *Candida* isolation had a mean CD4 of 22%, while those with negative colonization (N=12) had a mean CD4 of 21%, indicating that both groups had moderate immunosuppression.

The DMFT/DMFTS and dmft/dmfs scores and teeth with dentine carious lesions scores are shown in Table 2. There was a significant statistical correlation between the mean dentinal carious lesion score and positive *Candida* isolation for both groups (P=<.05, Mann-Whitney test; (Figure 1). This could not be demonstrated between the mean dmft/dmfs and DMFT/DMFS values in group 1 (P>.05, Mann-Whitney test), but was demonstrated between the mean values of DMFT/DMFS in group 2 (P<.05, Mann-Whitney test). When dentinal carious lesions were dichotomized as present or absent, group 1 patients who presented with caries activity were more colonized by *Candida* spp than group 2 (P<.05, Fisher test; Table 3).

Table2: DMFT/DMFTS and dmft/dmfs scores and teeth with dentinal carious lesions from

Indexes	Minimum		Maximum		Mean (± SD)	
	G1	G2	G1	G2	G1	G2
dmft	0.0	0.0	20.0	9.0	4.2 (±4.7)	2.0 (± 2.6)
dmfs	0.0	0.0	87.0	20.0	11.4 (±15.6)	5.1 (± 6.1)
DMFT	0.0	0.0	9.0	5.0	1.4 (± 2.0)	0.9 (± 1.3)
DMFS	0.0	0.0	16.0	8.0	2.6 (± 4.2)	1.5 (± 2.3)
Dentinal Caries Teeth	0.0	0.0	19.0	8.0	2.0 (±3.5)	1.5 (± 2.1)

HIV-infected children (n=62) and their seronegative siblings (n=40).

Table 3: Association between presence of dentinal carious lesions and Candida sp isolation in HIV-infected children (n=62) and their seronegative siblings (n=40).5

Candida SP isolation	Negative	Positive	Negative	Positive
	G1	G1	G2	G2
Presence of dentinal carious lesions	3 (4.8%)[a]	31 (50.0%)[a]	5 (12.5%)	15 (37.5%)
Absence of dentinal carious lesions	9 (14.5%)	19 (30.7%)	12 (30.0%)[b]	8 (20.0%)[b]

a- P<0.05 (Fisher's test)

b- P>0.05 (Chi-square test)

Table 4: DMFT/DMFTS and dmft/dmfs scores and teeth with carious lesions of 6 patients with oral candidiasis.

Indexes	Minimum	Maximum	Mean (± SD)
dmft	6.0	20.0	10.5 (± 5.1)
dmfs	12.0	87.0	34.7 (± 27.6)
DMFT	0.0	4.0	0.8 (± 1.6)
DMFS	0.0	5.0	1.0 (±2.0)
Dentinal Caries Teeth	1.0	19.0	7.5 (± 6.2)

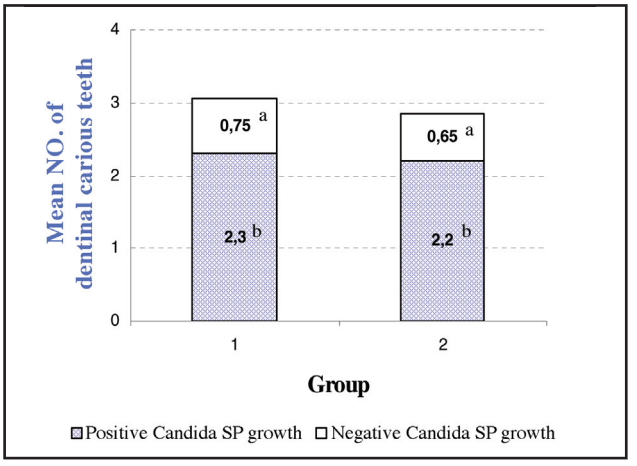

Fig.1. Relation of Candida SP growth and mean values of teeth with dentinal caries lesions in HIV-infected children (N=62) and their seronegative siblings (N=40; different letters: P=<.05, Mann-Whitney test).

A positive significant correlation between the number of carious teeth and *Candida* cfu was observed in group 1 (P<.05, r=0.324, Spearman test) and group 2 (P<.05, r= 0.554; Figure 2).

Oral examination revealed 6 patients with oral candidiasis: (a) 1 presenting as pseudomembranous; (b) 2 presenting with erythematous candidiasis; and (c) 3 presenting with angular ohueilities. All patients had active dentinal caries lesions. Of those: 4 subjects had AIDS and severe immunosuppression; and 5 had positive growth (1 with moderate and 4 with heavy growth). The mean DMFT/DMFS and dmft/dmfs scores and the number of dentinal caries lesions were higher when compared to with the mean scores of the total sample (Tables 2 and 4).

DISCUSSION

The prevalence of *Candida* spp in the oral cavity of HIV-infected children is considered significant in predicting the evolution of AIDS in these patients. A study by Flaitz et al[15] (1998) obtained a very similar result: from all saliva analyzed, around 80% of the HIV-infected children were colonized by *Candida* spp. Hicks et al[16] (1998), however, in a cytologic analysis of saliva of HIV-infected pediatric population, observed that only 19% of the subjects had fungal organisms detected. Of those, only 22% were symptomatic HIV-infected children, which may explain the lower percentage of patients with positive isolation.

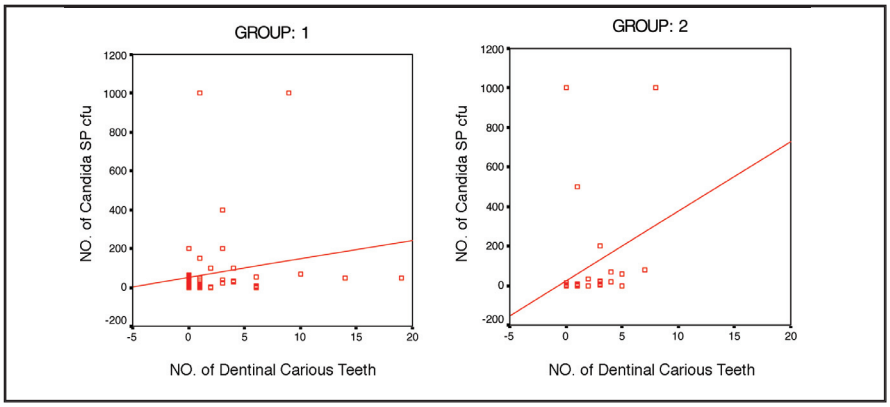

Fig.2. Correlation between Candida SP cfu and number of dentinal carious lesions teeth isolation in HIV-infected children (N=62) and their seronegative siblings (N=40).

Some studies have demonstrated an association between carious teeth and *Candida* spp colonization.[25,27,28] Russell et al (1990)[33] observed that *Candida* counts in a group of 372 Scottish children were consistently and significantly associated with caries prevalence, as either DS or DMFS scores. Sziegoleit et al (1999)[26] could isolate *Candida* species from the saliva of 67% of Hungarian adolescents with active caries. Only one child (2%) of 49 caries-free subjects, however, presented positive candidal growth. Moreover, considering those with active caries, 90% of them showed no positive growth for fungal organisms after dental treatment. Similar results were also observed in a group of Brazilian children after dental stabilization procedures (atraumatic restorative treatment [ART]), in which a reduction of 70% and 46% was demonstrated, respectively, on *Candida* counts when using zinc oxide-eugenol and glass ionomer cements.[28]

In the present study, no statistical difference was observed between dmft/dmfs and DMFT/DMFS scores and positive mycotic cultures in HIV-infected children, except for DMFT/DMFS scores in seronegative children. Coulter et al (1993)[34] did observe the same results when studying a group of adolescents: those with higher mean DMFS scores presented detectable levels of *Candida* comparing to *Candida*-free children. Gabris et al (1999)[35] also found statistically significant correlations between DMFT/DMFS mean values and salivary *Candida* spp counts among adolescents. When the number of subjects' teeth with dentinal carious lesions (or dichotomizing the presence caries activity) was evaluated in the present research, however—separately from the filling (f/F) and missed (m/M) scores—a significant finding was observed. Thirty-one group 1 children (50%) with dentinal carious teeth had *Candida* isolates present in their saliva, while only 3 (5%) with dentinal caries presented negative isolation (Table 3). This suggested a statistical significance (P=<.05, chi-square test) between *Candida* colonization and dentinal carious teeth only for group 1 patients. This agrees with the results of another study, in which there was a statistical significant difference, demonstrating that 69% of children with caries and 5% who were caries-free were found to be *Candida* carriers.[36]

In this study, a positive and statistical correlation between the number of dentinal carious teeth and *Candida* counts (ufc/ml) was demonstrated in both groups. Similar results were noticed by Coulter et al (1993)[34]: the mean DMFS score was higher in the group with higher *Candida* counts, but the correlation between caries experience and *Candida* levels was not statistically significant.

Jacob et al (1998)[25] found a relationship between candidal colonization of dentinal carious lesions and the presence of oral candidiasis in HIV-infected adults. Although the candidal colonization of carious teeth had not been performed in the present study, it was observed that 6 patients exhibited oral candidiasis during the examination and all of them had active dentinal caries (5 children had at least 4 teeth with dentinal carious lesions). In addition, the mean dentinal caries score was much higher (7.5) in relation to the mean score (2.0) of all subjects, indicating that dentinal carious lesions can be considered a risk factor not only for candidal colonization, but also for oral candidiasis in HIV-infected children.

It should also be determined whether the presence of fungal organisms in active carious lesions may be secondary to a preceding or existent oral candidiasis or if OC may be secondary to carious lesions[25]—once the latter can be considered a protective niche and work as an oral reservoir for fungal organisms. Therefore, the importance of oral health maintenance in HIV-infected children should be stressed by implementing health programs and eliminating all dentinal carious lesions by restoration or stabilization procedures in order to remove all possible candidal niches and, consequently decrease the oral prevalence of *Candida* spp and the risk of developing oral candidiasis.

Considering the risk factors for the oral prevalence of *Candida* spp, it was verified that immunosuppression did not influence the detection of this fungal organisms in the oral cavity of the HIV-infected children of the present study once moderate immunosuppression was present in those with or without positive growth.

It is well known that oral candidiasis: is an opportunistic infection; may be of great morbidity; and is considered a marker of immunosuppression for HIV-infected patients. Consequently, attention should be paid to strategies that reduce this lesion's opportunistic etiological agent: *Candida* spp. The results of this research demonstrate that dentinal carious lesions may be associated with oral *Candida* isolation; and, therefore be considered a risk factor for oral colonization in HIV-infected children.

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