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Salivary IgA to cariogenic bacteria in HIV-positive children and its correlation with caries prevalence and levels of cariogenic microorganisms

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The interrelationship of HIV infection, dental caries and mucosal immune responses remains controversial. In our study population of 40 HIV-infected and 40 healthy control children (ages 2–5 years) there was a significantly higher prevalence of dental caries in HIV-infected children ($P<0.05$). The extent of caries correlated with the severity of HIV disease. To determine whether the immunosuppression that ensues after HIV infection could contribute to the increased caries prevalence, the concentrations of total IgA and IgA specific to cariogenic bacteria (*Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus acidophilus*) were determined in whole saliva by enzyme-linked immunosorbent assay . Levels of the same bacteria were also quantified in saliva using checkerboard DNA–DNA hybridization. A significantly increased level of total salivary IgA was found in the HIV-positive population ($P < 0.05$), but there were comparable titers of specific IgA to cariogenic bacteria in HIV-positive and healthy controls. The microbiological assessment also demonstrated similar levels of cariogenic microorganisms in both groups. We conclude that HIV-positive children appear to maintain the capacity to mount a mucosal immune response to cariogenic microorganisms, at least until late stages of disease.

Key words: dental caries; HIV infection; secretory IgA

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The literature supports the concept that there is a higher prevalence of dental caries in HIV-infected children than in noninfected children (6, 13, 16, 17, 34, 35, 44, 47). According to Howell et al. (21), caries prevalence in HIV-infected children is very high, mainly in the primary dentition. In their study, almost 50% of HIV-infected children had extensive decay, with at least 10 affected surfaces. In a well-designed study using noninfected siblings as controls to compensate for confounding factors, Madigan et al. (27) found an average dmft of 3.8 for HIV-positive children vs. only 1.5 for control siblings. Several hypotheses have been proposed to justify such findings, e.g.:

- a higher sugar consumption by these patients, as an attempt to increase caloric intake and compensate for weight loss (20);
- the sucrose content in medications (16, 21);
- alterations in salivary flow as a result of drug ingestion or as a consequence of HIV salivary gland disease (15, 25, 28, 37, 38);
- immunodeficiency resulting from the HIV infection (27, 45).

HIV infection results in a progressive decrease in the levels of $CD4^+$ T lymphocytes. Since this cell has a pivotal role in the maturation of the secretory immune system, it is expected that this system would be altered in these patients. IgA is the main

immunoglobulin in saliva, and a drop in its levels could impact on caries development. The impact of HIV infection on salivary IgA levels is unclear, with reports of both decreased (28, 31, 41) and increased titers (2, 18). Sweet et al. (41) have reported that despite raised titers of serum IgA, HIV-infected patients presented with reduced salivary IgA concentrations. In contrast, Grimoud et al. (18), described increased salivary IgA levels in HIV-positive subjects when $CD4^+$ cells were <200/ml. The literature is sparse concerning specific IgA to bacteria associated with oral infections, but most data suggest raised levels in HIV-positive patients, possibly reflecting the increased degree of infection. Higher levels of IgA antibodies to *Candida* species have been reported in HIV patients, including AIDS patients, compared to sero-negative controls (10). Challacombe & Sweet (8) described raised titers of salivary antibodies to cells of *S. mutans* in HIV-positive and AIDS patients. Nevertheless, studies of the impact of HIV infection on the levels of salivary IgA in children, and its correlation with caries prevalence are, to our knowledge, still lacking.

The purpose of the present investigation was as follows:

- to determine the caries prevalence in pediatric patients infected with HIV compared to uninfected children;
- to compare the levels of caries-associated microorganisms (*Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus acidophilus*) in the saliva of HIV-infected children to the levels found in the control group (3);
- to compare the levels of total IgA and IgA specific to the aforementioned bacteria in whole saliva from the test and control groups;
- to evaluate the interrelationship of these parameters to the clinical and immunologic status of the HIV-positive children.

Materials and methods

Study populations

The study population was composed of 80 subjects (40 HIV-infected children and 40 control subjects) with a mean age of 4.36 years (± 0.08). There were 37 females and 43 males. There was no difference between the HIV-positive and HIV-negative groups with respect to age or gender (Table 1). The 40 HIV-infected children were selected from a group of HIV-positive children attending the Pediatric AIDS Outpatient Clinic of the Pediatric Institute Matagão Gesteira, Rio de Janeiro, Brazil, and the 40 controls were selected from a group of patients from the Pedodontic Department of the Federal University of Rio de Janeiro. Both centers are located in the same geographic region of the city of Rio de Janeiro and, hence, provide service to the same districts. Both populations also had a similar socioeconomic background. The HIV-positive group had a definitive diagnosis of HIV infection according to criteria established by the Centers for Disease Control and Prevention (7), and was examined between March 2000 and March 2001. Children in the control group had no clinical signs of immunodeficiency and were not using any drug at the time of the investigation. Parental informed consent was obtained for all patients before they were examined. The study protocol was approved by the ethics committee of the Federal University of Rio de Janeiro.

Table 1. Mean age (in years) of the subjects in the study, and their distribution according to group and sex

	Age	Sex		
Group	Mean ± SEM	M (%)	F (%)	*n*
HIV	4.33 ± 0.16	23 (57.5%)	17 (42.5%)	40
Control	4.34 ± 0.07	20 (50.0%)	20 (50.0%)	40

Data were obtained from patient medical records regarding:

- the clinical and immunologic classification;
- clinical manifestations of HIV infection;
- medications and laboratory parameters, including CD4 and CD8 lymphocytes, and T4/T8 ratio and viral load.

The data regarding the systemic condition of the HIV-positive subjects were the most recent available in the patient's medical chart at the time of the oral examination. However, the maximum interval between the oral exam and the quantification of laboratory data was 3 months.

Caries index

Caries prevalence was evaluated using the dmft (d = decayed, m = missing, f = filled, t = teeth) and dmfs (d = decayed, m = missing, f = filled, s = surfaces) indexes according to WHO criteria (48). In addition to the identification of decayed, missing and filled teeth and surfaces, the presence of white spot lesions (wsl) was also registered and analyzed separately. The diagnostic criteria for carious and white spot lesions were those used in studies of Carvalho et al. (5), and Bjorndal et al. (4).

Saliva collection and processing

Stimulated saliva was collected from every child at least 1 h after the last meal. The initial saliva produced after 1 min of paraffin chewing was discarded. The subjects then chewed for an additional 5 min, during which time whole saliva was collected. Very young children had samples collected using a suction bulb (Sigma. São Paulo. SP). For those children, the chewing of the bulb tip served as the salivary flow stimulus. Saliva samples clearly contaminated with blood were discarded. The samples were immediately placed on ice and transferred to the laboratory for processing. An aliquot of 100 µl from each sample was transferred to an Eppendorf tube (Sigma, São Paulo, Brazil) and frozen at −20°C for microbiological analysis. The remainder of the sample was placed in an Eppendorf tube, clarified by centrifugation at 10,000 × *g* for 15 min at 4°C and frozen at −20°C for enzyme-linked immunosorbent assay (ELISA). To control for serum and gingival crevicular fluid (GCF) contamination of the saliva samples, an ELISA kit specific for transferrin (Salimetrics LLC, State College, PA) was used. The transferrin concentration in mg/dl for each saliva sample tested was determined by converting its optical density (OD) value using the formula:

$$OD = cx^b.$$

Microbiological assessment

The presence and levels of three caries-related species (*S. mutans, S. sobrinus and L. acidophilus*) were determined by a modification of the checkerboard DNA–DNA hybridization method described by Socransky et al. (39). In brief, bacteria in 100 µl of frozen saliva (see above) were lysed by the addition of 0.5 M NaOH and the DNA denatured through boiling. The pH of the samples was neutralized by the addition of 800 µl of 5 M ammonium acetate. Single stranded DNA was pipetted in individual lanes on a nylon membrane (Boehringer Mannheim, Indianapolis, IN) using a checkerboard slot blot device (Minislot 30, Miniblotter 45, Immunetics, Cambridge. MA). DNA deposited onto the membrane was fixed by exposure to ultra-

violet light source (Stratolinker®, Stratagene, La Jolla, CA). Digoxigenin-labeled whole genomic DNA probes were prepared for each of the reference strains (*S. mutans* [ATCC 25175]; *S. sobrinus* [Forsyth Institute Collection] and *L. acidophilus* [ATCC 314]) using a random primer technique (14). The probes and hybridization buffer were combined and loaded onto the membrane containing the DNA from the saliva samples, using the Miniblotter, after rotating the membrane 90°, and hybridized at 70°C. Bound probes were detected using phosphatase-conjugated antibody to digoxigenin and chemiluminescence (Boehringer Mannheim). Signals were evaluated visually by comparison with the standards at 10^5 and 10^6 bacterial cells for the test species on the same membrane. Signals were recorded as: 0 – not detected; 1 – $<10^6$ cells/ml; 2 – 10^6 cells/ml; 3 – $>10^6$ and $<10^7$ cells/ml; 4 – 10^7 cells/ml and 5 – $>10^7$ cells/ml. The sensitivity of this assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Quantification of total IgA

The amount of total IgA was determined by ELISA. In brief, microplates (Costar, Corning, Acton, MA) were coated with goat antihuman α chain purified antibodies (Calbiochem®, San Diego, CA), diluted at 1/500 in buffer I (sodium carbonate. pH 9.6 with 0.2% sodium azide) and incubated. Plates were then blocked with 0.1% bovine albumin in buffer II (phosphate-buffered saline [PBS] with 0.05% Tween 20 and 0.02% NaN_3) for 30 min and incubated with diluted saliva samples (1/1600 in buffer II with 0.1% bovine albumin). The plates were incubated with the primary antibody (rabbit anti-human α chain) (Sigma, St. Louis, MO) diluted in buffer II with 0.1% albumin (1/1600) for 2 h and bound primary antibody was detected by reaction with goat anti-rabbit IgG conjugated to alkaline phosphatase diluted in buffer II (1/20,000) (Sigma). Substrate (p-nitrophenylphosphate [1 mg/ml]; Sigma) diluted in buffer IV (0.05 M sodium carbonate, pH 9.8, with $MgCl_2$) was added to each well and after 30 min the reaction was stopped by the addition of 1 N NaOH. Conversion of substrate was determined at OD_{405} using an ELISA reader (BIO-TEK Instruments, Inc., Winooski, VT). The plates were washed three times with 0.9% NaCl, 0.05% Tween 20 following each step. Unless otherwise stated, all incubation times were 2 h and all reactions took place on a rocking platform at room temperature.

Salivary antibody concentrations were calculated by reference to pooled standard saliva obtained from 10 control patients with high levels of antibody activity. A standard dilution curve ranging from 1 : 200 to 1 : 6400 was assayed with every microtiter plate. To calculate the concentration of IgA in the pooled saliva in μg/ml, a commercial kit specific for salivary secretory IgA (SIgA) (Salimetrics LLC, State College, PA) was used. The IgA concentration in μg/ml for each saliva tested was determined by comparing its OD value to the linear portion of the standard curve and using the formula:

$$OD = c\ (Log_n\ x) + b.$$

Measurement of antibodies to cariogenic bacteria in saliva

A modified ELISA was used to determine the levels of specific antibodies to caries-associated bacteria. In brief, microplates (Costar) were coated with formalin-killed microorganisms: *S. mutans* (ATCC 25175); *S. sobrinus* (Forsyth Institute Collection); *L. acidophilus* (ATCC 314) in PBS at 3×10^7 organisms/ml, and incubated for 3 h at room temperature. After at least 2 days of incubation at 4°C, the plates were washed and incubated with diluted saliva samples (1/400 in buffer II with 0.1% bovine albumin) followed by incubation with the primary antibody (rabbit anti-human α chain) (Sigma) diluted in buffer II with 0.1% albumin (1/500). Detection of bound primary antibody and conversion of substrate were done in the same manner as for total IgA.

Salivary antibody concentrations were calculated by reference to pooled standard saliva obtained from 10 control patients with high levels of antibody activity. The standard saliva was assigned an arbitrary value of 100 Units for a 1/400 dilution and a standard dilution curve ranging from 1 : 100 to 1 : 3200 was assayed with every microtiter plate. The OD data generated from this standard curve were plotted on a semilogarithmic scale and a standard curve was generated by linear regression. The IgA antibody level for each saliva tested was determined by comparing its OD value to the linear portion of the standard curve and using the formula:

$$OD = c\ (Log_n\ x) + b.$$

Statistical analysis

Data were entered into databases using software specific for biostatistics (SYSTAT® 10.2). Comparisons between means were done using the Mann–Whitney test. Comparisons between subgroups were sought using analysis of variance (ANOVA) and regression analysis. For all analyses a *P*-value of less than 0.05 was considered significant.

Results

Clinical and immunologic characteristics of the HIV-positive population

The clinical and immunologic classifications of the HIV-infected children (7) are presented in Fig. 1. In general there was good correlation between the immunologic and clinical classifications. Most patients in the study (26/40) could be described as having AIDS (classification C and/or 3). The caries experiences of the HIV-positive and control populations are shown in Fig. 2. As indicated, the HIV-positive children had more caries compared to healthy controls, on both the dmft and dmfs scales. The differences between HIV-positive and HIV-negative children were

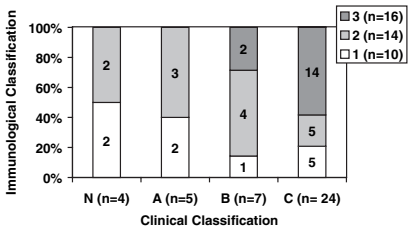

Fig. 1. HIV-infected children were clinically classified as: N = lack of symptoms, A = mild symptoms, B = moderate symptoms or C = AIDS-associated diseases; and immunologically as: 1 = lack of immunodeficiency, with CD4 levels >1000, and 25%; 2 = mild immunodeficiency, with CD4 levels between 500 and 999, and 15–24%; 3 = severe immunodeficiency, with CD4 levels <500, and 15%. According to criteria established by the CDC (7).

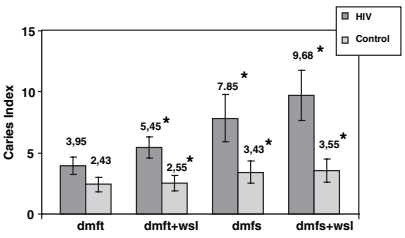

Fig. 2. Mean values of dmft, dmf+wsl, dmfs and dmfs+wsl in HIV-positive children ($n = 40$) and control patients ($n = 40$). Standard error of the mean is given in brackets. *$P < 0.05$, Mann–Whitney test. dmft: d = decayed, m = missing, f = filled, t = teeth; dmfs: d = decayed, m = missing, f = filled, s = surface and wsl = white spot lesions.

even greater when white spot lesions were taken into consideration. These findings are consistent with previous studies that show increased caries incidence in HIV-infected children (6, 13, 16, 17, 34, 35, 44, 47).

HIV status and dental caries experience

The relationship between HIV status and caries experience was then examined. As seen in Fig. 3, there was a linear relationship between the severity of HIV clinical symptoms and the extent of caries using all four caries indices. These relationships were statistically significant for the indices that quantified the presence of white spot lesions ($P = 0.01$ and $p = 0.02$ for dmft+wsl and dmfs+wsl, respectively), when evaluated using a regression of the caries indexes means against the clinical categories (converted to scores 0, 1, 2 and 3). The data confirm the clear trend toward more dental caries with the progression of HIV infection. A similar relationship was observed when the immunologic status of the HIV-infected children was correlated with caries indices. The most immunocompromised children (classification 2 and 3) showed a trend toward elevated caries experience (dmfs+wsl = 8.86 and 10.13, respectively) when compared to children with immune classification 1 (dmfs+wsl = 5.25), although the results did not reach statistical significance.

Relationship between HIV infection and salivary levels of cariogenic microorganisms

The levels of the cariogenic microorganisms *S. mutans*, *S. sobrinus* and *L. acidophilus* in saliva from HIV-positive and HIV-negative children are summarized in Table 2. The distribution of cariogenic microorganisms was similar in the two groups, although the levels of all three microorganisms were below 10^6/ml in more HIV-positive children.

Table 2. Distribution of microbiological scores in HIV-positive ($n = 39$) and control ($n = 40$) children. Scores were assigned as: 0 – not detected; 1 – $<10^6$ cells/ml; 2 – 10^6 cells/ml; 3 – $>10^6$ and $<10^7$ cells/ml; 4 – 10^7 cells/ml and 5 – $>10^7$ cells/ml

	Level of microorganisms						
Bacterium	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	*n*
S. mutans							
HIV	10 (25.6)	14 (35.9)	5 (12.8)	6 (15.4)	3 (7.7)	1 (2.6)	39*
Control	3 (7.5)	13 (32.5)	9 (22.5)	13 (32.5)	1 (2.5)	1 (2.5)	40
S. sobrinus							
HIV	11 (28.2)	13 (33.3)	8 (20.5)	4 (10.3)	2 (5.1)	1 (2.6)	39*
Control	3 (7.5)	5 (12.4)	14 (35.0)	17 (42.5)	1 (2.5)	–	40
L. acidophilus							
HIV	13 (33.3)	11 (28.2)	7 (17.9)	7 (17.9)	1 (2.6)	–	39*
Control	4 (10.0)	14 (35.0)	9 (22.5)	7 (17.5)	6 (15.0)	–	40

*The microbiological assessment could not be performed in one HIV-positive child.

Salivary IgA and specific IgA to cariogenic microorganisms

HIV-infected children presented with significantly higher levels of total salivary IgA than the HIV-negative controls (51.9 μg/ml ± 3.2 and 45.6 μg/ml ± 2.6, respectively [mean ± standard error of the mean]) (Fig. 4). However, there was no difference between the two groups in levels of specific IgA to *S. mutans, S. sobrinus* or *L. acidophilus* (Fig. 5). There was also no statistically significant difference between the levels of salivary IgA among the HIV-positive children, classified according to their clinical manifestations. However, a small trend towards lower levels in the asymptomatic children, followed by higher IgA levels in the groups with mild and moderate symptoms and a return to lower levels in the AIDS group was noted (Fig. 6). A similar trend was seen with respect to the immunologic classification. Children without immunodeficiency and with CD4 levels >1000 (score 1) showed lower levels of antibodies (both total and specific) than children with mild immunodeficiency (score 2). However, HIV-positive children with severe immunodeficiency (CD4 levels <500 − score 3) had lower levels than the group with mild immunodeficiency. These data suggest that HIV infection does not have a major impact on salivary IgA responses to cariogenic bacteria.

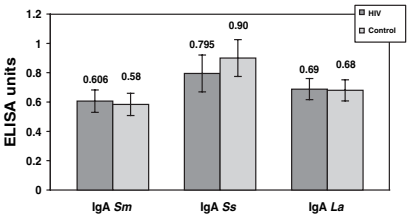

Fig. 5. Concentrations in ELISA units of IgA antibodies to *S. mutans* (*Sm*); *S. sobrinus* (*Ss*) and *L. acidophilus* (*La*), in the saliva of HIV-positive children ($n = 40$) and control patients ($n = 40$). Standard error of the mean is given in brackets.

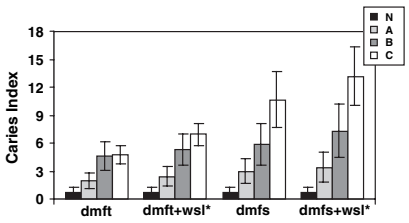

Fig. 3. Mean values of dmft, dmft+wsl, dmfs and dmfs+wsl in HIV-positive children ($n = 40$), distributed according to their clinical classification: N = lack of signs and symptoms, A = mild symptoms, B = moderate symptoms, C = AIDS-associated diseases (N [$n = 4$]; A [$n = 5$]; B [$n = 7$] and C [$n = 24$]). Standard error of the mean is given in brackets. *$P < 0.05$, regression of means against clinical classification, converted to scores: 0, 1, 2 and 3.

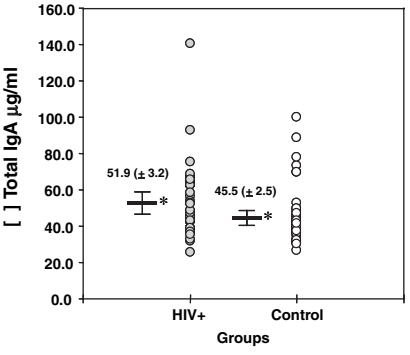

Fig. 4. Concentration in μg/ml of total IgA antibodies in the saliva of HIV-positive children ($n = 40$) and control patients ($n = 40$). Bars and brackets indicate mean and standard error of the mean, respectively. *$P < 0.05$, Mann–Whitney test.

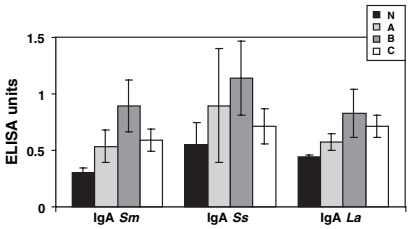

Fig. 6. Concentrations in ELISA units of IgA antibodies to *S. mutans* (*Sm*); *S. sobrinus* (*Ss*) and *L. acidophilus* (*La*) in the saliva of HIV-positive children ($n = 40$) distributed according to their clinical classification: N = lack of signs and symptoms, A = mild symptoms, B = moderate symptoms, C = AIDS associated diseases (N [$n = 4$]; A [$n = 5$]; B [$n = 7$] and C [$n = 24$]). Standard error of the mean is given in brackets.

Transferrin concentration in saliva samples

Leakage of blood and GCF into the oral cavity may compromise the measurement of salivary components (43). In order to estimate the level of contamination of the saliva samples by potential extraoral sources of IgA, the levels of transferrin were calculated for each sample. Transferrin is a large protein found in great quantities both in blood and in GCF, but it is present in minute amounts in saliva. The results demonstrated that both groups presented only trace amounts of transferrin in saliva: 0.187 mg/dl ± 0.031 and 0.167 mg/dl ± 0.035 (mean ± standard error of the mean) for the HIV-positive and control groups, respectively. There was also no significant difference between the groups (P-value = 0.52 using the Mann–Whitney test). Therefore, we conclude that the contamination of serum and GCF in our saliva samples was only marginal.

Discussion

HIV infection profoundly affects host immune responses, resulting in opportunistic infections secondary to immunosuppression. In the present case control study of 2–5-year-old children, we found a statistically significant increase in the prevalence of caries in HIV-infected children, with the strongest relationships with incipient caries ('white spot lesions').

Most studies of the dental caries status of HIV-infected children have demonstrated a higher prevalence of caries in is group compared to healthy subjects (6, 13, 16, 17, 34, 35, 44, 47). Such a discrepancy was also observed by Hicks et al. (19) when his data for HIV-infected children were compared with indexes from national surveys in the US. Children between the ages of 2 and 4 had a dmft of 2.7 in his study, in contrast to a national mean of 1.2 for this age group. Other findings, such as those reported by Madigan et al. (27), also corroborate our results. In their study, children between 3 and 6 years of age presented a dmfs of 8.2, while their uninfected siblings had a dmfs of only 4.8.

In search of the causes of such a high prevalence of dental caries in HIV-infected subjects, several authors have correlated this finding with the high sugar content present in their medications. In the treatment of HIV-infected children, the medication is often mixed with sugar solutions and given in a nursing bottle. According to Roberts & Roberts (36), liquid, sweet medications are capable of inducing caries irrespective of other dietary factors and due to their constant use, children with chronic medical disorders are at risk for developing dental caries as a result of the treatment for their primary condition. HIV-infected children most definitely fall into this category. Despite not being the purpose of our work to investigate the correlation between the sugar content of medications and caries experience of HIV-infected children, it must be pointed out that 85% of the test group was under antiretroviral therapy, most of which was administered via a syrup vehicle. Hence, a possible contribution of the sugar content in medications cannot be ruled out.

Reduced salivary flow may also increase caries risk. Several medications utilized in the treatment of HIV infection such as didanosine, and the presence of hypertrophy of the parotid gland (a condition also associated with HIV infection), may reduce the production of saliva by infected children, elevating their chances of developing dental caries (15, 25, 28, 38). In the present study, 53% of infected children were receiving didanosine and 5% of the test group had salivary gland alterations. Although we did not determine salivary flow rates, the use of didanosine by the majority of HIV-infected children in our sample, coupled with the presence of salivary gland enlargement in a proportion of these patients, may have also influenced the caries risk in this group.

HIV infection is one of the conditions associated with nutritional deficiency, resulting from the oral lesions that are associated with HIV infection and which directly affect food ingestion (1). To overcome this problem, physicians often recommend a hypercaloric diet (21). Since most of our sample was composed of children with frank AIDS, it is likely that they were on a hypercaloric regimen, which may also increase their caries risk. In their work, Madigan et al. (27) could not demonstrate a higher sugar intake by their study population of HIV-infected children, when compared with healthy siblings, even when the use of medications was taken into account. Nevertheless, his sample was composed of relatively healthy patients, most presenting good general health despite the HIV infection. Hence, their need for nutritional supplements was probably lower than in the present study group. On the other hand, their findings suggest that other mechanisms were responsible for the elevated caries risk in the HIV-infected children.

The immunologic deficiency resulting from the progression of HIV infection has been reported as a risk factor for dental caries in children by several authors (6, 19, 27, 45, 47). Valdez et al. (45) were the first to correlate caries prevalence with the immune deficiency of HIV-infected patients, reporting that the most systemically compromised children presented with more decay. This association between the immune status and caries risk was also suggested by Madigan et al. (27), who failed to find differences in sugar intake and levels of cariogenic bacteria (*S. mutans* and *Lactobacillus*) that could explain a higher prevalence of caries. In the present study, it was seen that caries prevalence increased with the worsening of the clinical condition of the HIV-positive children. When categorized according to their clinical status, the average dmft, dmft+wsl, dmfs and dmfs+wsl of the test group increased gradually with the evolution of the infection, with children with moderate and severe clinical symptoms presenting scores much higher than those with mild symptoms or without manifestations (Fig. 3). This trend could be confirmed by regression analysis of dmft+wsl and dmfs+wsl against the clinical classifications, demonstrating a statistically significant correlation ($P < 0.05$). Madigan et al. (27) also reported that children at a more advanced disease stage had a higher caries prevalence. A similar trend could be detected within the immune classification; patients with moderate to severe immunosuppression presented a higher caries prevalence than patients without immunosuppression. In a previous report, our group has also described a trend towards clinically and immunologically compromised young patients (up to 5 years old) presenting with more caries (6). In this regard, Hicks et al. (19) found an increased number of carious lesions with the decline in the percentage of $CD4^+$ cells and Vieira et al. (47) have reported an increased incidence of caries in children with a CD4 : CD8 ratio of less than 0.5, when compared to children with a ratio greater or equal to 0.5.

In light of the pivotal role of $CD4^+$ cells in the maturation of the mucosal immune system, and the fact that HIV infection leads to a decrease in the numbers of such cells, it is expected that secretory immunity, including salivary IgA, would be compromised with the progression of HIV infection. Sweet et al. (40) found that HIV-infected individuals had elevated levels of serum immunoglobulins, whereas salivary IgA levels were reduced. However, a reduction

in the salivary flow of the HIV-infected subjects may have impacted on the salivary IgA levels in that study. Indeed, the same group could not confirm these observations in a later study, in which antibody titers to *Candida albicans* and to *S. mutans* were increased in whole and parotid saliva from HIV patients. Atkinson et al. (2) also reported elevated levels of salivary IgA, consistent with the well-established rise in systemic immunoglobulins that follows HIV infection. Such findings conflict with the immunosuppression that ensues the infection by HIV, raising questions about the function of such antibodies.

In our study, total salivary IgA was quantified in whole saliva, collected after stimulation with paraffin chewing. This form of saliva collection may increase the contribution of nonsalivary IgA, arising from the circulation through the GCF and lesions present in the oral cavity. This is particularly relevant in our study, since the HIV-infected children are more susceptible to intraoral lesions such as gingivitis, linear gingival erythema and candidiasis, and increased levels of serum IgA have been found in AIDS patients (22, 41, 42). To account for the possible contribution of nonsalivary sources of IgA, we quantified transferrin in our samples. This large protein is present in large quantities in plasma and GCF, but minimally present in saliva (11). Our data clearly demonstrated that transferrin was only present in trace amounts in both groups and, more importantly, there was no significant difference in the levels of transferrin between the two groups. Hence, it is unlikely that serum contamination of our samples had an impact on the findings reported here.

HIV-infected children presented total salivary IgA levels that were significantly higher than in children in the control group. However, there were no differences with respect to specific IgA to cariogenic bacteria. Myint et al. (32), reported similar findings of higher levels of total IgA in HIV-positive patients. This increased immune reaction is most probably a response to the higher level of antigens present in the oral cavity of these patients, who suffer from opportunistic infections such as candidiasis (8, 10, 12). Sweet et al. (40) found that antibody titers to cells of *C. albicans* were correlated with the presence of candidiasis, indicating an antibody response to antigenic challenge despite the HIV infection. Mandel et al. (28) also failed to find reductions in the IgA titers in HIV-infected subjects. It would seem that, in general, HIV-positive children maintain an intact immune response capacity to a number of antigens presented to the mucosal immune system. It is possible that the level of $CD4^+$ helper activity, although reduced, is still sufficient to induce normal mucosal responses to oral microorganisms in HIV-infected individuals, at least until the later stages of disease. This is supported by our observation that HIV children with the most severe disease had lower IgA levels than those with mild or moderate disease (Fig. 6). In addition, it is possible that compensatory mechanisms for IgA production may be in place in these patients, such as polyclonal B-cell activation (8).

This observation, coupled with the tendency for clinically compromised HIV-positive children to have more dental caries, suggests that this increased salivary IgA concentration is a result of infection, instead of filling a protective role. The decrease in humoral response, noted in the progression from HIV infection to AIDS, despite an increased prevalence of dental caries, suggests that the compensatory response is overcome with progressive immunodeficiency induced by the HIV virus. Justifying the high bacterial burden of mutans streptococci and lactobacilli found in their sample of HIV-infected children, Madigan et al. (27) suggested that lack of early salivary exchange between HIV-positive patients and their caretakers would delay the initiation and development of a protective salivary IgA response. They also implied that the high levels of contamination could result from a loss of immune response to caries-associated flora as the disease progresses.

Coogan et al. (10) also reported comparable findings when quantifying anti-*Candida* IgA in the secretions from the parotid gland. They reported that the amount of IgA was lower in AIDS patients than in HIV-infected subjects and concluded that this could be an indicator of immunosuppression and a marker for the progression of HIV infection to AIDS. Studying responses to a pneumococcal vaccine in both serum and saliva, Mascart-Lemone et al. (29) found that both serum and saliva IgA responses were depressed in patients with CD4 counts of less than 500. Challacombe & Sweet (8) confirmed such findings, working with whole saliva and parotid saliva from HIV-infected patients. In their study, the levels of IgA to *Candida* and *S. mutans* were raised for patients infected with HIV but reduced in AIDS patients, indicating that the immune response was being compromised by the evolution of the HIV infection.

HIV-infected patients may have a reduced salivary flow rate (15, 25, 28, 38). Alterations in salivary flow may increase the concentration of its constituents, despite an overall lower output (23, 46). We did not normalize our saliva samples based on flow due to the difficulties of making this measurement in the field. It is therefore possible that differences in salivary flow rate between the groups could be responsible for the higher concentration of total IgA in the HIV-infected group. Nevertheless, an investigation into the effects of early HIV infection in salivary function not only confirmed our findings of increased concentration of total salivary IgA, but also showed that the only salivary component displaying an increased secretory rate was secretory IgA (26). Furthermore, not every study has reported a reduced salivary flow in HIV-infected patients (31, 33). Even so, a possible impact of a reduced flow rate in the saliva of HIV-positive children cannot be ruled out, and our findings should be interpreted with this caveat in mind.

Despite a higher prevalence of dental caries in HIV-infected children, the quantification of cariogenic microorganisms (*S. mutans, S. sobrinus* and *L. acidophilus*) showed a similar level of colonization by these microorganisms. Madigan et al. (27) verified that children infected with HIV had higher levels of *S. mutans* and *Lactobacillus* than their HIV-negative siblings. However, when only the data for the younger children (3–6 years old) were analyzed, the number of patients with more than 10^6 colony-forming units of *S. mutans* was higher for the test group. These findings contrast with those presented by Hicks et al. (19), who observed that the immunosuppression and the decline in CD4 cells could elevate the number of cariogenic bacteria in saliva and, consequently, in the dental plaque of HIV-positive pediatric patients. Their results demonstrated that the levels of *S. mutans* and *Lactobacillus,* as well as the caries prevalence, was significantly correlated with the time of HIV infection, a factor associated with the severity of the disease.

It remains difficult to explain the presence of higher levels of incipient decay in the HIV group, despite similar levels of caries-associated microorganisms and specific IgA to these bacteria in both groups. As noted above, Madigan et al. (27) also failed to find a higher level of infection by *S. mutans* in an HIV cohort, when younger subjects were considered separately. There are several potential explanations for these observations. First, the SIgA antibodies produced in HIV-infected children may be of lower avidity than those produced in

healthy subjects (2). Millon et al. (30) demonstrated that HIV-infected patients could produce high levels of specific salivary antibodies to *Candida* antigens; however, these antibodies were not efficient in limiting candidiasis. Second, other elements of the immune system, in particular molecules such as lactoferrin and lysozyme, may also be compromised in the saliva of HIV patients (3, 9).

In conclusion, the presence of increased titers of salivary IgA specific to cariogenic bacteria, associated with high levels of cariogenic microorganisms and an elevated prevalence of caries in HIV-infected children, reveals that they maintain the capacity to mount a mucosal immune response. However, in advanced stages of the disease (AIDS), this system shows signs of compromise. Despite the presence of very high levels of colonization by cariogenic microorganisms and carious lesions, there is a decrease in the level of response, measured by the quantification of IgA.

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References

1. American Dietetic Association. Position of the American Dietetic Association: oral health and nutrition. J Am Diet Assoc 1996: **96**: 184–189.
2. Atkinson JC, Yeh C, Oppenheim FG, Bermudez D, Baum BJ, Fox PC. Elevation of salivary antimicrobial proteins following HIV-1 infection. J Acquir Immune Defic Syndr 1990: **3**: 41–48.
3. Bard E, Laibe S, Clair S, Biichle S, Millon L, Drobacheff C, et al. Nonspecific secretory immunity in HIV-infected patients with oral candidiasis. J Acquir Immune Defic Syndr 2002: **31**: 276–284.
4. Bjorndal L, Larsen T, Thylstrup A. A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment intervals. Caries Res 1997: **31**: 411–417.
5. Carvalho JC, Thylstrup A, Ekstrand KR. Results after 3 years of non-operative occlusal caries treatment of erupting permanent first molars. Community Dent Oral Epidemiol 1992: **20**: 187–192.
6. Castro GF, de Souza IP, Oliveira RH, Portela MB, Esteves C. [Prevalence of caries and its correlation with clinical and immunological classification in HIV-infected children]. Pesqui Odontol Bras 2001: **15**: 91–97.
7. CDC. CDC 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. MMWR 1994: **43**: 1–19.
8. Challacombe SJ, Sweet SP. Salivary and mucosal immune responses to HIV and its co-pathogens. Oral Dis 1997: 3 (Suppl. 1): S79–S84.
9. Challacombe SJ, Sweet SP. Oral mucosal immunity and HIV infection: current status. Oral Dis 2002: **8** (Suppl. 2): 55–62.
10. Coogan MM, Sweet SP, Challacombe SJ. Immunoglobulin A (IgA), IgA1, and IgA2 antibodies to *Candida albicans* in whole and parotid saliva in human immunodeficiency virus infection and AIDS. Infect Immun 1994: **62**: 892–896.
11. Curtis MA, Sterne JA, Price SJ, Griffiths GS, Coulthurst SK, Wilton JM, et al. The protein composition of gingival crevicular fluid sampled from male adolescents with no destructive periodontitis: baseline data of a longitudinal study. J Periodontal Res 1990: **25**: 6–16.
12. Drobacheff C, Millon L, Monod M, Piarroux R, Robinet E, Laurent R, et al. Increased serum and salivary immunoglobulins against *Candida albicans* in HIV-infected patients with oral candidiasis. Clin Chem Lab Med 2001: **39**: 519–526.
13. Eldridge K, Gallagher JE. Dental caries prevalence and dental health behaviour in HIV infected children. Int J Paediatr Dent 2000: **10**: 19–26.
14. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983: **132**: 6–13.
15. Fox PC. Salivary gland involvement in HIV-1 infection. Oral Surg Oral Med Oral Pathol 1992: **73**: 168–170.
16. Gehrke FS, Johnsen DS. Bottle caries associated with anti-HIV therapy. Pediatr Dent 1991: **13**: 73.
17. Gelbier M, Lucas VS, Zervou NE, Roberts GJ, Novelli V. A preliminary investigation of dental disease in children with HIV infection. Int J Paediatr Dent 2000: **10**: 13–18.
18. Grimoud AM, Arnaud C, Dellamonica P, Lodter JP. Salivary defence factor concentrations in relation to oral and general parameters in HIV positive patients. Eur J Oral Sci 1998: **106**: 979–985.
19. Hicks MJ, Flaitz CM, Carter AB, Cron SG, Rossmann SN, Simon CL, et al. Dental caries in HIV-infected children: a longitudinal study. Pediatr Dent 2000: **22**: 359–364.
20. Howell RB, Houpt M. More than one factor can influence caries development in HIV-positive children. Pediatr Dent 1991: **13**: 247–.
21. Howell RB, Jandinski J, Palumbo P, Shey Z, Houpt M. Dental caries in HIV-infected children. Pediatr Dent 1992: **14**: 370–371.
22. Kozlowski PA, Jackson S. Serum IgA subclasses and molecular forms in HIV infection: selective increases in monomer and apparent restriction of the antibody response to IgA1 antibodies mainly directed at env glycoproteins. AIDS Res Hum Retroviruses 1992: **8**: 1773–1780.
23. Kugler J, Hess M, Haake D. Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. J Clin Immunol 1992: **12**: 45–49.
24. Laibe S, Bard E, Biichle S, Vielle J, Millon L, Drobacheff C, et al. New sensitive method for the measurement of lysozyme and lactoferrin to explore mucosal innate immunity. Part II. time-resolved immunofluorometric assay used in HIV patients with oral candidiasis. Clin Chem Lab Medical 2003: **41**: 134–138.
25. Leggott PJ, Robertson PB, Greenspan D, Wara DW, Greenspan JS. Oral manifestation of primary and acquired immunodeficiency diseases in children. Pediatr Dent 1987: **9**: 98–104.
26. Lin AL, Johnson DA, Stephan KT, Yeh CK. Alteration in salivary function in early HIV infection. J Dent Res 2003: **82**: 719–724.
27. Madigan A, Murray PA, Houpt M, Catalanotto F, Feuerman M. Caries experience and cariogenic markers in HIV-positive children and their siblings. Pediatr Dent 1996: **18**: 129–136.
28. Mandel ID, Barr CE, Turgeon L. Longitudinal study of parotid saliva in HIV-1 infection. J Oral Pathol Med 1992: **21**: 209–213.
29. Mascart-Lemone F, Levy J, Depelchin S, Dehennin JP, Cooman R, Sibille Y, Vaerman JP. IgA subclasses and molecular size in children with vertically transmitted HIV infection. Adv Exp Med Biol 1995: **371B**: 1007–1010.
30. Millon L, Drobacheff C, Piarroux R, Monod M, Reboux G, Laurent R, Meillet D. Longitudinal study of anti-*Candida albicans* mucosal immunity against aspartic proteinases in HIV-infected patients. J Acquir Immune Defic Syndr 2001: **26**: 137–144.
31. Muller F, Holberg-Petersen M, Rollag H, Degre M, Brandtzaeg P, Froland SS. Nonspecific oral immunity in individuals with HIV infection. J Acquir Immune Defic Syndr 1992: **5**: 46–51.
32. Myint MM, Steinsvoll S, Odden K, Dobloug J, Schenck K. Salivary IgA responses to bacteria in dental plaque as related to periodontal and HIV infection status. Eur J Oral Sci 1997: **105**: 562–570.
33. Pollock JJ, Santarpia RP III, Heller HM, Xu L, Lal K, Fuhrer J, et al. Determination of salivary anticandidal activities in healthy adults and patients with AIDS. a pilot study. J Acquir Immune Defic Syndr 1992: **5**: 610–618.
34. Pongsiriwet S, Iamaroon A, Kanjanavanit S, Pattanaporn K, Krisanaprakornkit S. Oral lesions and dental caries status in perinatally HIV-infected children in Northern Thailand. Int J Paediatr Dent 2003: **13**: 180–185.
35. Ribeiro AA, Portela M, Souza IP. [Relation between biofilm, caries activity and gingivitis in HIV + children]. Pesqui Odontol Bras 2002: **16**: 144–150.
36. Roberts IF, Roberts GJ. Relation between medicines sweetened with sucrose and dental disease. Br Med J 1979: **2**: 14–16.
37. Schiodt M, Greenspan D, Daniels TE, Nelson J, Leggott PJ, Wara DW, et al. Parotid gland enlargement and xerostomia associated with labial sialadenitis in HIV-infected patients. J Autoimmun 1989: **2**: 415–425.
38. Schiodt M, Greenspan D, Levy JA, Nelson JA, Chernoff D, Hollander H, et al. Does

HIV cause salivary gland disease? AIDS 1989: **3**: 819–822.
39. Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE. 'Checkerboard' DNA-DNA hybridization. Biotechniques 1994: **17**: 788–792.
40. Sweet SP, Cookson S, Challacombe SJ. *Candida albicans* isolates from HIV-infected and AIDS patients exhibit enhanced adherence to epithelial cells. J Med Microbiol 1995: **43**: 452–457.
41. Sweet SP, Rahman D, Challacombe SJ. IgA subclasses in HIV disease: dichotomy between raised levels in serum and decreased secretion rates in saliva. Immunology 1995: **86**: 556–559.
42. Sweet SP, Rahman D, Challacombe SJ. Serum and saliva immunoglobulin A concentrations show an inverse relationship in HIV infection and AIDS. AIDS 1995: **9**: 1288–1289.
43. Tabak LA. A revolution in biomedical assessment: the development of salivary diagnostics. J Dent Educ 2001: **65**: 1335–1339.
44. Tofsky N, Nelson EM, Lopez RN, Catalanotto FA, Fine DH, Katz RV. Dental caries in HIV-infected children versus household peers: two-year findings. Pediatr Dent 2000: **22**: 207–214.
45. Valdez IH, Pizzo PA, Atkinson JC. Oral health of pediatric AIDS patients: a hospital-based study. ASDC J Dent Child 1994: **61**: 114–118.
46. van der Reijden WA, van der Kwaak JS, Veerman EC, Nieuw Amerongen AV. Analysis of the concentration and output of whole salivary constituents in patients with Sjogren's syndrome. Eur J Oral Sci 1996: **104**: 335–340.
47. Vieira AR, de Souza IP, Modesto A, Castro GF, Vianna R. Gingival status of HIV^+ children and the correlation with caries incidence and immunologic profile. Pediatr Dent 1998: **20**: 169–172.
48. World Health Organization. Oral health surveys: basic methods, **Vol. 3**. Geneva: WHO, 1987.