Scientific Article

Use of Chlorhexidine Gel (0.2%) to Control Gingivitis and Candida Species Colonization in Human Immunodeficiency Virus-infected Children: A Pilot Study

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Abstract: *Purpose: The purpose of this study was to evaluate chlorhexidine to control gingivitis and Candida species (spp.) in children infected with the human immunodeficiency virus (HIV) and their acceptance of the therapy. Methods: Twenty-six HIV+ children were selected, and oral exam-established biofilm, gingival indexes, and stimulated saliva were collected for Candida ssp. identification. The children brushed their teeth for 21 days with chlorhexidine gel (0.2%). Salivary samples, biofilm, and gingival indexes were collected after 21-days and again 35 days after ceasing gel use. The children answered a questionnaire about the therapy. Results: All children tested positive for Candida and gingivitis. After 21 days, Candida counts and gingivitis decreased in 25 and 26 children, respectively. Mean reduction was approximately 68% for Candida spp. and 74% for gingivitis. Thirty-five days after discontinuing gel use, gingivitis and Candida spp. increased in 13 and 16 patients, respectively. Considering the Candida spp., the heavy growth was lower in the first re-evaluation. Candida albicans was the most frequent species. Approximately 85% did not experience inconvenience with the gel, and approximately 48% thought it was good for tooth-brushing. Conclusion: Chlorhexidine therapy may be an option to treat and prevent gingivitis and reduce yeast counts in children infected with HIV. (Pediatr Dent 2011;33:153-7) Received September 8, 2009 / Last Revision November 19, 2009 / Accepted December 8, 2009*

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Children infected with the human immunodeficiency virus (**HIV**) often present with oral manifestations such as oral candidiasis, herpes simplex infection, parotid enlargement, recurrent aphthous ulcers, and periodontal alterations.[1-6] Among these lesions, candidiasis, gingival linear erythema, and gingivitis are considered to be the most prevalent.[7-11]

Immunocompromised pediatric patients seem to have more severe bacterial infections, including gingivitis and periodontitis, than healthy children.[6] Also, candidiasis is a fungal infection and can cause significant morbidity for such patients, leading to odynophagia, dysphagia, malnutrition, and systemic infections.[12] Treatment of candidiasis generally involves azole and polyene compounds,[13] and the frequent use of these antifungal products may create resistant Candida species (**spp.**).[14,15] Furthermore, systemic antifungal therapy is generally associated with side effects and drug interactions.[16] Therefore, an effective topic treatment that could prevent and control lesions such as candidiasis and gingivitis would help improve the quality of life for these children.

Chlorhexidine (**CHX**) is the most widely used antimicrobial agent to control dental biofilm and recurrence of gingivitis.[17,18] Topically applied CHX has been reported to have an antifungal effect in the oral cavity,[19] and it may also be used for the prevention and treatment of oral candidiasis in children and adults infected with HIV.[16,20] Therefore, the aim of the present study was to evaluate the efficacy of daily tooth-brushing with chlorhexidine gel (0.2%) over a 21-day period in children infected with the human immunodeficiency virus.

Methods

Patient population. We conducted an experimental study without a control group. Twenty-six 6- to 13-year-old HIV+ patients were selected by convenience from a cohort of patients at the Rio de Janeiro Federal University pediatric hospital, Rio de Janeiro, Brazil. The dental appointments were conducted on the same day and place of medical appointments due to the difficulties in scheduling these patients for dental examination. Inclusion criteria were the presence of gingivitis and positive culture of Candida spp. in salivary samples. The definitive HIV+ diagnosis was confirmed by 2 positive enzyme-linked immunosorbant assay (ELISA) and a positive Western blot tests. Children with carious lesions or teeth that required extraction were excluded,[21] as well as children under antifungal treatment or those wearing orthodontic or prosthetic appliances and those with behavioral or

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cognitive deficiencies. This study was approved by the research ethics committee of the Federal University of Rio de Janeiro and informed consent was obtained from the legal guardians of all participants.

Data collection. A detailed medical history, including the use of antiretroviral therapy and laboratory data (CD$_4$%, T$_4$/T$_8$, and viral load), was obtained from the child's medical records. At the first exam (baseline), each child received a detailed clinical examination performed by a qualified professional for determining the biofilm index and gingival bleeding index, according to Ribeiro et al (2002).[22] Examinations were performed with the use of a mirror, millimeter drill (Hu-Friedy), and gauze for viewing, drilling, and drying, respectively. After probing, the gingival index was determined and the percentage of provoked or spontaneous bleeding sites for each child was recorded. Next, stimulated saliva samples were obtained by asking the patients to chew a paraffin stick (1 g) for 5 minutes. All samples were kept refrigerated, and laboratory procedures were performed within 2 hours.

CHX gel instructions. All selected children received a kit consisting of a toothbrush and tube of 0.2% CHX gel (Perioxidin Gel, Lacer, SA, Barcelona, Spain) for home use. Children were instructed to brush their teeth with the gel for 1 minute twice a day over 21 days. Patients were told not to use fluoride dentifrice or dental floss during this period. After 21 days, patients stopped using CHX and returned to fluoride dentifrices.

Evaluations. Biofilm index, gingival index, and stimulated saliva samples were taken: at the study's commencement (baseline); 21 days after using CHX gel (first re-evaluation); and 35 days after suspending CHX gel use (second re-evaluation). At the first re-evaluation, the children answered a questionnaire to determine any possible side effects and their perception of the acceptability of the CHX therapy. Questions

regarding flavor, foam, residual taste, and dental stains also were answered, and response options were "good," "acceptable," and "bad."

Laboratory and statistical analysis. Saliva was diluted at a 1:10 ratio with 0.9% sterile saline solution (pH 7.2). Aliquots of 100 μl were cultured on plates with chromogenic agar (CHROMagar Candida, Probac, São Paulo, Brazil) and incubated at 37° C for 48 to 72 hours, which enables a presumptive identification of Candida spp. by evaluating the color of each colony formed.[23]

For statistical analysis, the extent of gingivitis was classified as "local" (≤30% of the examined sites with gingivitis) or "generalized" (>30% of examined sites with gingivitis).[24] Also, plates with positive growth were classified according to Lamey et al.[25] as "mild" (<10 CFU/ml), "moderate" (11-50 CFU/ml), and "heavy growth" (>50 CFU/ml). All data were descriptively and statistically analyzed via Epi Info 3.04 (World Health Organization, Geneva, Switzerland/Centers for Disease Control and Prevention, Atlanta, Ga) and SPSS 11.0 (SPSS Inc, Chicago, Ill) using Wilcoxon, Kruskal-Wallis, Fisher, and Spearman's correlation tests with a 5% level of significance.

Results

The children's mean age (N=26) was 10.7 years old (±1.58 SD), and 15 (56%) were female. Regarding laboratory exams, the mean CD4% was 23% (±12.8), approximately 63% of subjects had T$_4$/T$_8$ more than 0.5, and only 1 had undetectable viral load. Approximately 18% received only reverse transcriptase inhibitor medication, and approximately 74% were treated with highly active antiretroviral therapy (**HAART**). Antiretroviral treatment regimens from the start to the end of the study were unchanged, and laboratory data also remained constant throughout the entire period. All children presented with positive culture for Candida spp. and clinical signs of gingivitis at baseline. Also, no child presented with oral candidiasis or other oral manifestations associated with HIV infection.

The percentage of biofilm index at both re-evaluations showed there was a reduction of thick biofilm (indices 3-5) throughout the study when compared to the baseline (*P*<.01, Wilcoxon test).

Mean percentage (%) of sites with gingivitis and the number of Candida spp. colony-forming units (**CFU**) at

Table 1.   GINGIVAL CONDITION AND NUMBER OF COLONY-FORMING UNITS (CFU) OF CANDIDA SPP. OF THE CHILDREN AT EACH EVALUATION

Parameter	Mean values (±SD) in children			*P*-value
	Baseline (N=26)	First re-evaluation (N=26)	Second re-evaluation (N=18)	Kruskall-Wallis
% of sites with gingivitis	23.53 (±20.50)	7.53 (±7.24)	15.24 (±12.52)	<.001
Candida spp. (CFU/ml)	134.5 (± 177.4)	47.38 (±80.30)	95.16 (±129.32)	.03
	Frequency of children			
	Baseline	First re-evaluation	Second re-evaluation	Fisher's test
Gingivitis:				
Yes	26	18	15	<.01[†]
No	-	8	3	
Extension*:				
Local	16	18	14	<.01[†]
Generalized	10	-	1	

* Local=30% of sites with gingivitis; generalized=>30% of sites with gingivitis.[23]
† *P*-value between baseline and first re-evaluation.

Table 2.   PERCENTAGE OF CHILDREN WITH CANDIDA SPP. ACCORDING TO GROWTH TYPE AT EACH EVALUATION

	Percentage of children		
Candida spp. growth*	Baseline (N=26) N (%)	First re-evaluation (N=26) N (%)	Second re-evaluation (N=18) N (%)
Mild	5 (19)	13 (50)	7 (39)
Moderate	8 (31)	5 (19)	2 (11)
Heavy	13 (50)	8 (31)	9 (50)

*Mild=<10 CFU/ml; moderate=11-50 CFU/ml; heavy=>51 CFU/ml.[23]

each evaluation are shown in Table 1. After 21 days of CHX use (first re-evaluation), there was a significant reduction in gingivitis among all children ($P<.01$, Kruskal-Wallis); 9 children experienced a 100% reduction. The mean reduction was approximately 74%. At the second re-evaluation (35 days after stopping CHX), there was a loss of 8 children from the sample. Among the remaining children, gingivitis increased in 13, but values were less when compared to the baseline. Also during the study, the number of patients with generalized gingivitis decreased significantly ($P<.01$, Fisher test). The drop out were 8 patients probably due to financial difficulties to attend the appointment, the absence of symptoms following CHX use or the little importance given to oral health within the larger scope of general health.

Additionally, Candida spp. counts (CFU/ml) decreased significantly during the study (Table 1). The reduction occurred in 25 patients in the first re-evaluation, with a 100% reduction in 7 children. The mean reduction was approximately 68%. At the second re-evaluation, 1 child presented with pseudo-membranous candidiasis, and Candida spp. counts increased in 16 patients vs the 21-day exam. According to the classification of Candida spp. growth types (Table 2), there was a nonsignificant decrease in heavy growth in the first re-evaluation when compared to the baseline.

Table 3. PERCENTAGE OF CHILDREN WITH CANDIDA ACCORDING TO THE DIFFERENT SPECIES AT EACH EVALUATION			
	Frequency of subjects		
Candida spp.	Baseline (N=26) N (%)	21 days (N=26) N (%)	56 days (N=18) N (%)
Candida albicans	25 (93)	18 (67)	16 (84%)
Candida tropicalis	6 (22)	4 (15)	4 (22%)
Candida parapsilosis	1 (4)	1 (4)	–

Candida albicans was the most frequently isolated species at the baseline and both re-evaluations. Candida tropicalis and Candida parapsilosis also were identified, but in much lower numbers (Table 3). No correlation was observed between gingivitis or Candida spp. reduction regarding the use of antiretroviral therapy and CD4% ($P=.09$, Spearman's correlation).

Side effects and treatment acceptance. Approximately 85% of the children reported that they did not experience any inconvenience regarding the use of CHX gel. Figure 1 shows the classification of the gel in terms of flavor, foam, residual taste after tooth-brushing, and dental staining according to the children. Most children mentioned that they enjoyed the flavor of CHX gel: 48% of them thought that it was good, and 44% rated it as acceptable. Three children (11%), however, reported dental staining, and 1 child (7%) complained about the flavor.

Discussion

Our study reported a 73% reduction in gingivitis after toothbrushing with CHX gel twice a day for 21 days at home. Gingivitis reduction occurred simultaneously with biofilm reduction; the percentage of thick biofilm was approximately 74% at baseline and dropped to 52% at the end of the 21-day period. Our results are similar to other studies that also found satisfactory results in children and adolescents with or without systemic alterations using this substance combined with mechanic control.[18,26-31] No literature study was found, however, that confirmed the effect of this antimicrobial agent on gingivitis control in HIV+ children.

This study's limitations included the modest sample size and the absence of a control group, which was not possible due to the ethics committee's restrictions. Also, because it was difficult for these patients to come to dental appointments, they were not chosen randomly. Therefore, any comparison of our results must be made with caution and should be evaluated in accordance with the population studied as well as the vehicle, time period of use, and CHX concentration.

Since the introduction of HAART in 1996, which includes protease inhibitors for treating HIV infection, a reduction in the prevalence of the oral manifestations in infected patients has been observed, including oral candidiasis. Such lesions, however, are still very frequent.[11,32] Systemic or topical antifungal substances, such as nystatin and fluconazole, are generally the treatment of choice for these lesions. Fungi may become resistant to this therapy, however, and, even when responsive, lesions may recur soon after such treatment ends.[13]

CHX's efficacy regarding Candida spp. also was evaluated. This substance is known to have an antifungal effect and decreases the adherence of fungus, thereby reducing the start of infection.[13] Barasch et al.[16] carried out a pilot study to evaluate the efficacy of a CHX rinse in the treatment and prevention of oral candidiasis in HIV+ children and observed a significant reduction in the CFU/ml of Candida spp. In our study, the use of a CHX gel for tooth-brushing spread its effect throughout the oral cavity; consequently, this had a topical effect on oral mucosa, causing a decrease in Candida spp. Despite the fact that the presence of micro-organisms does
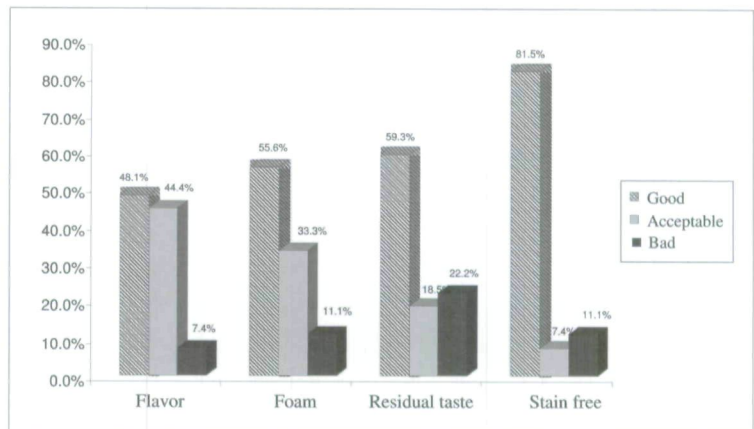

Figure 1. Children's perceptions of flavor, foam, residual taste, and staining of chlorhexidine gel.

not necessarily imply the occurrence of lesions, there is indeed an association between colonization and clinical manifestations.[33] In immunocompromised patients, who present a susceptibility to the development of candidiasis, this therapy may prevent such lesions, with the added advantage of being free of drug interactions.[16]

Similar to other reports,[16,34,35] *C albicans* was the most frequently observed species throughout this study. *C tropicalis* and *C parapsilosis* also were observed, but at much lower frequencies and without phenotypical identification, making it difficult to perform an analysis of species resistance and selection. Therefore, further studies are needed to clarify this issue.

One of CHX's advantages is its substantiality, allowing retention in the oral cavity and a residual effect for over 12 hours, depending on the dosage and vehicle. In a clinical study, Edmilson et al.[36] found that the return to the initial microbiological condition occurred 2 to 8 weeks after interruption of the use of CHX gel, depending on the vehicle. The present study conducted a 35-day re-evaluation after interruption of CHX use to assess the time elapsed before the child's oral health returned to the baseline situation. Even with a sample loss of approximately 30% (8 patients), a 51% gingivitis increase was observed compared to the 21-day examination due to the loss of the antimicrobial effect. Ellopola and Samaranayake[19] reported that CHX's antifungal activity may be noticed even when it is administered with longer intervals due to its prolonged antifungal effect, thereby reducing patient exposure to the drug.

Similarly to what was observed with gingivitis, there was an increase of Candida spp. colonization in the saliva of the studied population 35 days after interrupting the CHX therapy. The time period for using CHX has not yet been completely established in the literature. The present study's results suggest the possibility of creating a protocol for using this substance, with an interruption period not less than 4 weeks. Patients should be re-evaluated individually and return to the therapy if necessary, considering that a 35-day interval is sufficient for an increase in the frequency of gingivitis and CFU/ml of Candida spp. among HIV+ children.

Despite the known side effects of this substance,[24,37,38] the use of CHX gel was well tolerated by the children, and most did not report any side effects, although 3 complained of tooth staining. Abreu et al.[18] also found satisfactory acceptance of CHX gel for home use in patients with disabilities. Barasch et al.[16] reported that, despite the satisfactory tolerance by patients to a CHX rinse, the high noncompliance rate to the treatment was a study limitation. Furthermore, although the authors were unaware of the reasons, they suggest that this is a common problem among HIV-infected populations. Although it was expected that side effects would lead to a decrease in compliance to treatment, the main reasons for the drop outs found in this study were financial difficulties; the absence of symptoms following CHX use; and the little importance given to oral health within the larger scope of general health.

Finally, this study's results may be quite valuable in preventing oral infections in HIV+ children and treating gingivitis. During the 35-day period without CHX use, there was an increase in gingivitis and Candida spp. growth. Therefore, the reduction observed in gingivitis and the Candida spp. count during the gel use period was mainly due to CHX's antimicrobial effect. Thus, this therapy proved effective and demonstrated good acceptance in the present study for the control of gingivitis and colonization by Candida spp. in HIV+ children.

Conclusions
Based on this study's results, the following conclusions can be made:

1. There was a significant reduction in gingivitis and in Candida species counts after tooth-brushing with chlorhexidine gel twice a day for 21 days at home.
2. Chlorhexidine therapy may be an option for the treatment and prevention of oral candidiasis and gingivitis in children infected with the human immunodeficiency virus.

Acknowledgments

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