
The effect of HAART on salivary microbiota in the Women's Interagency HIV Study (WIHS)

Mavash Navazesh, DMD,^a Roseann Mulligan, DDS, MS,^b Janice Pogoda, PhD,^c Deborah Greenspan, BDS, DSc,^d Mario Alves, DDS, MS, DSc,^e Joan Phelan, DDS,^f John Greenspan, BDS, PhD,^g and Jorgen Slots, DDS, DMD, PhD, MS, MBA,^h Los Angeles and San Francisco, Calif, Chicago, Ill, and New York, NY
UNIVERSITY OF SOUTHERN CALIFORNIA, UNIVERSITY OF CALIFORNIA,
UNIVERSITY OF ILLINOIS, AND NEW YORK UNIVERSITY

Objective. Study the prevalence of potentially pathogenic microorganisms in saliva of HIV-positive women in the Women's Interagency HIV Study.

Study design. 157 HIV-positive and 31 HIV-negative women were studied. At baseline and every 6 months over 4 years, information was collected on socioeconomic and educational status, oral and systemic health, including HIV markers and antiretroviral therapy, and frequency of professional oral care utilization. Bacterial and yeast pathogenic isolates from stimulated whole saliva were tentatively identified using standard methodologies.

Results. The prevalence of microorganisms in stimulated saliva of HIV-positive women was not significantly different from that of HIV-negative women. In HIV-positive women, highly active antiretroviral therapy (HAART) was independently and significantly associated with the presence of a variety of salivary bacterial species. HAART increased the risk for recovering *Fusobacterium* species ($P < .001$), enteric gram-negative rods ($P < .05$), *Peptostreptococcus micros* ($P < .05$), *Campylobacter* species ($P < .0001$), *Eubacterium* species ($P < .001$), and *Tannerella forsythia* ($P < .01$). In contrast, HAART led to decreased recovery rate of yeasts (*Candida albicans* and *Candida dubliniensis*) ($P < .0001$).

Conclusion. The present findings suggest that the institution of HAART promotes an increasingly pathogenic salivary microbiota, at least temporarily. Similar findings have been reported for various nonoral microbial ecosystems.

(*Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;100:701-8)

HIV-1 infection per se and in conjunction with anti-retroviral therapy has significant impact on the oral mucosa and salivary gland structure and function.¹⁻⁸ A

significantly higher prevalence of xerostomia (the subjective complaint of dry mouth) and salivary gland hypofunction (the objective evidence of reduced saliva secretion) has been described in HIV-positive compared to at-risk HIV-negative participants of the Women's Interagency HIV Study (WIHS).^{9,10} Since 1980, studies also have documented an enhanced susceptibility of HIV-infected patients to oral yeast, bacterial, and viral infections.¹¹⁻¹⁷ Recently, an increased occurrence of oral warts, salivary gland enlargement, and dry mouth has been associated with highly active antiretroviral therapy (HAART) as part of a new phenomenon called immune restoration or reconstitution disease (IRD).¹⁸⁻²² The impact of HIV infection on salivary electrolytes (ie, sodium and chloride), and antimicrobial and antifungal proteins (ie, lysozyme, lactoferrin, secretory IgA, and histatin) is also well established.²³⁻²⁹ Less attention has been given to the salivary microbiota of HIV-infected patients. Saliva harbors 10^8 - 10^9 bacteria per mL,³⁰ which are derived from biofilms on the tongue and other mucosal surfaces, teeth, gingival crevices, and periodontal pockets.³¹

We have previously reported a significant association between HAART and salivary gland hypofunction in HIV-positive women belonging to this cohort of

^aAssociate Professor and Chair, Division of Diagnostic Sciences, School of Dentistry, University of Southern California, Los Angeles, California.

^bProfessor, Division of Health Promotion, Disease Prevention and Epidemiology; Associate Dean for Community Health, School of Dentistry, University of Southern California.

^cAssistant Professor, Department of Preventive Medicine, Keck School of Medicine, University of Southern California.

^dProfessor, Clinical Oral Medicine, Leland A. and Gladys K. Barber Distinguished Professor in Dentistry, Department of Orofacial Sciences, University of California, San Francisco.

^eClinical Professor, Periodontics, College of Dentistry, University of Illinois, Chicago.

^fProfessor and Chair, Department of Oral Pathology, Division of Biological Sciences and Surgery, New York University.

^gLeland A. and Gladys K. Barber Professor and Dean for Research School of Dentistry; Director, AIDS Research Institute, School of Medicine, University of California, San Francisco.

^hProfessor of Periodontology and Microbiology, School of Dentistry, University of Southern California.

Received for publication Aug 16, 2004; returned for revision Oct 4, 2004; accepted for publication Oct 8, 2004.

1079-2104/\$ - see front matter

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doi:10.1016/j.tripleo.2004.10.011

Table 1. Baseline demographic, socioeconomic, and oral health characteristics by HIV status

<i>Characteristics</i>		<i>HIV-positive women</i> (<i>n</i> = 157) <i>no. (%)</i>	<i>HIV-negative women</i> (<i>n</i> = 31) <i>no. (%)</i>	<i>P value</i> ¹
Age in years	<20	2 (1)	0 (0)	.92
	20-29	36 (23)	9 (29)	
	30-39	68 (43)	13 (42)	
	40-49	46 (29)	8 (26)	
	50+	5 (3)	1 (3)	
Race/ethnicity	Black	74 (47)	17 (55)	.73
	Hispanic	59 (38)	10 (32)	
	White	20 (13)	4 (13)	
	Other	4 (3)	0 (0)	
Education	Less than high school	78 (50)	8 (26)	.01
	At least high school	79 (50)	23 (74)	
Income per year	≤\$12,000	107 (71)	18 (62)	.35
	>\$12,000	44 (29)	11 (38)	
	Unknown	6	2	
HIV risk category	Intravenous drug use	62 (39)	11 (35)	.34
	Heterosexual contacts	58 (37)	8 (26)	
	Transfusion	7 (4)	2 (6)	
	Unidentified	30 (19)	10 (32)	
Current smoker	No	61 (39)	8 (26)	.17
	Yes	96 (61)	23 (74)	
Edentulous	No	147 (94)	29 (94)	.99
	Yes	10 (6)	2 (6)	
Regular dental visits	No	113 (72)	23 (74)	.80
	Yes	44 (28)	8 (26)	
% tooth surfaces with visible plaque	≤50%	65 (44)	13 (45)	.95
	>50%	82 (56)	16 (55)	
	Unknown	10	2	
% surfaces with gingival bleeding	≤10%	70 (48)	14 (48)	.97
	>10%	76 (52)	15 (52)	
	Unknown	11	2	
Any pocket depth >4 mm	No	113 (78)	22 (76)	.81
	Yes	32 (22)	7 (24)	
	Unknown	12	2	
% surfaces with attachment loss >2 mm	≤10%	60 (41)	16 (55)	.17
	>10%	85 (59)	13 (45)	
	Unknown	12	2	

¹Chi-squared test.

patients.³² The present study determined the presence of potentially pathogenic microorganisms in saliva, and evaluated the impact of oral, systemic and socioeconomic variables on the salivary microbiota of HIV-infected women. To our knowledge, this study represents the first longitudinal, gender-specific, controlled investigation of the salivary microbiota in HIV-infected individuals.

MATERIAL AND METHODS

Subjects

The WIHS was established in August 1993 to investigate the impact of HIV infection on women in the United States. Between October 1994 and November 1995, 2,058 HIV-positive and 568 HIV-negative women across the United States were enrolled in WIHS. The objectives, methodology, training, quality assurance,

and scope of activities involving the WIHS cohort are described elsewhere.^{33,34} Between April 1995 and October 1996, in addition to continuing medical evaluation, a subgroup of 157 HIV-positive and 31 HIV-negative women was randomly selected for study of pathologic changes of the salivary glands and oral tissues and of the microbiota of whole saliva. Study subjects received medical and oral examination at baseline and every 6 months.³⁴ This report is based on data collected at baseline and at 6-month follow-up evaluations over 4 years at 4 examination sites in Chicago, Los Angeles, New York, and San Francisco. All study participants consented to the research protocols that had been approved by appropriate institutional review boards. At each visit, participants received a comprehensive medical evaluation that included interviews and clinical and laboratory evaluations. The oral health evaluations included documentation of salivary gland function, mucosal lesions commonly associated with HIV-infection, coronal and root dental caries, and periodontal disease status. The number of teeth present, plaque index, gingival bleeding, periodontal pocket depth, and attachment loss were assessed according to established methods.³⁵ Quantification of HIV-1 RNA in plasma was performed by using the nucleic acid sequence-based application method (Organon Teknika, Durham, NC). At each visit, interviewers recorded self-reported antiretroviral use since the previous visit by inquiring about generic and brand drug names and by showing the participants photomedication cards. The antiretroviral therapy in WIHS is described elsewhere.³⁶ The definition of HAART followed the guidelines of the International AIDS Society—USA Panel Guidelines.^{37,38}

Microbiological study

Saliva stimulated by chewing for 2 minutes under standardized conditions³⁹ was collected in a graduated plastic collector containing 1.9 mL VMG I transport medium.⁴⁰ The salivary samples were placed in a plastic mailing container with ice packs and shipped with an overnight carrier to the Oral Microbiological Testing Laboratory at the University of Southern California School of Dentistry. Promptly after receiving samples, nonselective and selective bacterial and fungal isolation was carried out according to established procedures.⁴¹ Microorganisms were mechanically dispersed using a Vortex mixer at the maximal setting for 30 seconds, and then 10-fold serially diluted in VMG I medium. Using a sterile bent rod, 0.1 mL aliquots from 10³ to 10⁴ dilutions were plated onto nonselective 4.3% brucella agar (BBL Microbiology Systems, Cockeysville, Md) supplemented with 0.3% Bactoagar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells,

Table II. Prevalence of potential pathogens in the saliva of 157 HIV-positive and 31 HIV-negative women

Potential pathogens	HIV-positive, microbial pathogen-infected women no. (%)	HIV-negative, microbial pathogen-infected women no. (%)
<i>Fusobacterium</i> species	125 (80)	22 (71)
Yeast (<i>Candida</i> species)	108 (69)	23 (74)
<i>Peptostreptococcus micros</i>	107 (68)	22 (71)
<i>Campylobacter</i> species	95 (61)	21 (68)
<i>Eubacterium</i> species	73 (46)	19 (61)
<i>Tannerella forsythia</i>	65 (41)	12 (39)
<i>Prevotella intermedia</i>	42 (27)	12 (39)
<i>Porphyromonas gingivalis</i>	30 (19)	6 (19)
Beta-hemolytic streptococci	15 (10)	3 (10)
<i>Eikenella corrodens</i>	15 (10)	4 (13)
<i>Actinobacillus actinomycetemcomitans</i>	12 (8)	5 (16)
<i>Staphylococcus epidermidis</i>	4 (3)	0
<i>Staphylococcus aureus</i>	3 (2)	0
Enteric gram-negative rod-group (total)	61 (39)	16 (52)
<i>Pseudomonas aeruginosa</i>	32 (20)	3 (10)
<i>Escherichia coli</i>	18 (11)	4 (13)
<i>Enterobacter cloacae</i>	17 (11)	6 (19)
<i>Klebsiella pneumoniae</i>	16 (10)	8 (26)
<i>Acinetobacter</i> species	10 (6)	2 (6)
<i>Pseudomonas putida</i>	9 (6)	1 (3)
<i>Xanthomonas maltophilia</i>	9 (6)	0
<i>Klebsiella oxytoca</i>	8 (5)	4 (13)
<i>Citrobacter freundii</i>	8 (5)	1 (3)
<i>Enterobacter asburiae</i>	5 (3)	0

0.005% hemin, and 0.0005% menadione for determining total viable counts and proportions of specific species.⁴² 0.1 mL aliquots from 10-fold dilution of VMG I medium were plated onto TSBV medium⁴³ for culture of *Actinobacillus actinomycetemcomitans*, enteric gram-negative rods/pseudomonas, and yeasts. The brucella blood agar medium was incubated at 35°C in a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich) containing 85% N₂ 10% H₂ 5% CO₂ for 8 days. The TSBV medium was incubated in 10% CO₂ in air at 35°C for 4 days. Bacterial identification was performed according to methods described by Slots⁴¹ and by use of commercial micromethod systems. The microorganisms identified included *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia/Prevotella nigrescens* group, *Campylobacter* species, *Eubacterium* species, *Fusobacterium* species, *Peptostreptococcus micros*, *Eikenella corrodens*, *Staphylococcus* species, enteric gram-negative rods, β-hemolytic streptococci, and yeasts.

Table III. Multivariable odds ratios (OR) and 95% confidence intervals (CI) for associations between indicators of periodontal health, socioeconomic, dental healthcare utilization, and HIV therapy in HIV-positive women only¹

Independent variables	# Specimens: # Subjects:	<i>Fusobacterium</i> species	<i>Enteric rod</i>	<i>P. micros</i>
		(n = 276+, 274-) (n = 113+, 91-) OR (CI)	(n = 195+, 355-) (n = 94+, 108-) OR (CI)	(n = 168+, 382-) (n = 93+, 102-) OR (CI)
% surfaces with attachment loss >2 mm		1.6 (1.0-2.4)*	1.1 (0.7-1.7)	3.2 (1.9-5.3)****
High school graduate		0.6 (0.4-0.9)*	1.1 (0.7-1.6)	0.6 (0.4-1.0)*
Regular dental visits		0.8 (0.5-1.1)	1.2 (0.8-1.8)	0.8 (0.5-1.2)
Previously on HAART		1.4 (0.8-2.4)	2.1 (1.1-3.9)*	1.2 (0.6-2.3)
New on HAART		0.8 (0.4-1.6)	0.4 (0.2-1.0)	0.3 (0.1-0.9)*
HAART, not continuously		2.3 (1.1-5.1)*	1.0 (0.5-2.2)	1.4 (0.6-3.1)
HAART, continuously		2.4 (1.4-4.1)****	1.8 (1.1-2.9)*	1.8 (1.0-3.0)*

P* < .05.*P* < .01.****P* < .001.*****P* < .0001.¹Viral load, edentulous, no. of teeth, antibiotic, plaque, and gingival bleeding were included as covariates; for yeast, antifungal drug and pseudomembranous candidiasis were also included.²Zero positive specimens were "new on HAART"; therefore, "new on HAART" and "HAART, not continuously" were combined.

Statistical analysis

Chi-squared tests were used to compare baseline demographic, socioeconomic, and oral health characteristics of HIV-positive and HIV-negative women. For comparisons between HIV-positive and HIV-negative women on prevalence and levels of each test microorganism, the unit of observation was the subject. "Prevalence" was defined as ever having had a specimen that was positive for the microorganism over the length of the follow-up period. For each subject, the maximum level of each microorganism in the specimens collected over the length of follow-up was determined. Three levels of each pathogen were defined: "absent," "low," and "high," where the dividing point between "low" and "high" was based on the median maximum level among both HIV-positive and HIV-negative women. For analyses of prevalence and levels of individual microorganisms, maximum likelihood odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression; Fisher's exact test was used when zero cells prevented adjustment for number of specimens. Trend tests for microorganism levels used category levels as continuous variables. The Wilcoxon rank sum test was used to test for a difference in the mean number of specimens between HIV-positive and HIV-negative women.

For analyses of the effects of periodontal disease, socioeconomic status, dental healthcare utilization, and HAART on the presence of microorganisms in HIV-positive women, the unit of observation was the specimen, and OR and CI were calculated using logistic regression by generalized estimating equations with subject as a random effect.⁴⁴ Indicators of periodontal

disease were percent plaque, percent gingival bleeding, percent attachment loss, and any pocket depth >4 mm. Socioeconomic indicators were income, education, current smoking status, and number of sex partners since last visit. Self-reported HAART was classified as "never on HAART," "previously on HAART," "new on HAART (<12 months)," "on HAART not continuously for at least 12 months," and "on HAART continuously for at least 12 months." In addition, AIDS status, CD4, CD8, CD4/CD8 ratio, HIV-1 RNA level, being edentulous, number of teeth, use of antibiotic and antifungal drugs, number of oral lesions, and presence of pseudomembranous and erythematous candidiasis were included as covariates where appropriate. Analyses were limited to those pathogens that were present in at least 30 HIV-positive specimens. Continuous and ordinal variables were dichotomized, with the cutpoint based on the median value among HIV-positive specimens. Multivariable logistic regression was used to model independent variables simultaneously and to test for interactions between variables. Spearman or phi correlation coefficients were calculated for each pair of independent variables prior to multivariable analyses to assess the potential for multicollinearity.

Analyses of the effects of periodontal disease, socioeconomic status, and dental healthcare utilization on the presence of microorganisms in HIV-negative women were done as described above, except edentulousness and antibiotic use were not included as covariates, since too few observations were positive for these factors.

All significance tests were done at the .05 level. Analyses were carried out using SAS software, version 9 (SAS Institute, Cary, NC).

Table III. Continued

<i>Campylobacter</i> species (n = 146+, 404-) (n = 84+, 107-) OR (CI)	<i>Eubacterium</i> species (n = 85+, 465-) (n = 62+, 118-) OR (CI)	<i>T. forsythia</i> (n = 67+, 483-) (n = 52+, 121-) OR (CI)	<i>P. intermedia/ P. nigrescens</i> (n = 64+, 485-) (n = 36+, 133-) OR (CI)	<i>P. gingivalis</i> (n = 32+, 517-) (n = 26+, 137-) OR (CI)	Yeast (n = 262+, 288-) (n = 91+, 108-) OR (CI)
2.2 (1.3-3.9)**	4.7 (1.9-11.6)**	4.3 (2.0-9.2)***	0.7 (0.3-1.4)	3.3 (1.2-9.4)*	0.9 (0.5-1.5)
0.6 (0.3-0.9)*	0.7 (0.4-1.2)	0.7 (0.4-1.4)	0.6 (0.3-1.3)	1.4 (0.6-3.0)	1.1 (0.6-1.8)
0.6 (0.4-0.9)*	0.6 (0.3-0.9)*	0.8 (0.4-1.6)	0.9 (0.6-1.6)	0.6 (0.2-1.6)	0.7 (0.5-1.1)
4.1 (2.2-7.8)****	2.3 (1.1-4.9)*	2.5 (1.0-6.2)*	0.3 (0.1-1.0)*	2.3 (0.8-7.0)	0.4 (0.2-0.8)**
1.6 (0.7-3.6)	0.7 (0.2-2.2)	— ² —	0.7 (0.2-2.7)	0.4 (0.0-3.1)	1.2 (0.6-2.3)
1.4 (0.6-3.1)	1.1 (0.4-2.9)	0.8 (0.3-2.0)	0.4 (0.1-2.0)	2.7 (0.8-8.9)	0.7 (0.3-1.5)
7.2 (4.0-13.0)****	2.8 (1.5-5.1)***	3.1 (1.5-6.4)**	2.0 (0.9-4.1)	1.3 (0.5-3.4)	0.2 (0.1-0.4)****

RESULTS

HIV-positive and HIV-negative women were comparable on baseline demographic, socioeconomic, and oral health characteristics except for education (Table I). HIV-positive women had somewhat fewer specimens than HIV-negative women [mean (SD) = 4.4 (3.8) for HIV-positive, 5.7 (4.4) for HIV-negative; *P* = .11] and were less educated (*P* = .01); thus, education and number of specimens were used as covariates in comparisons between HIV-positive and HIV-negative women (as subject was the unit of observation).

Table II shows the prevalence of each microorganism by HIV status. Although none of the microorganisms was significantly more prevalent in HIV-positive than in HIV-negative women, ORs were >2.0 for *Citrobacter freundii* (OR = 3.3, CI = 0.4-28.8; *P* = .28), *Pseudomonas aeruginosa* (OR = 2.9, CI = 0.8-10.7; *P* = .11), and *Pseudomonas putida* (OR = 2.9, CI = 0.4-24.9; *P* = .32). There were no trends relating microorganism levels to HIV status (data not shown).

In multivariable analyses of the effects of socioeconomic, periodontal, oral health, and HAART on microorganism prevalence among HIV-positive specimens, education level, regularity of dental visits, attachment loss, and HAART consistently emerged as being associated with the presence or absence of specific microorganisms; the most important covariates appeared to be HIV-1 RNA, being edentulous, number of teeth, antibiotics, plaque index, and gingival bleeding, as well as, for yeast only, antifungal medications and pseudomembranous candidiasis. After adjusting for these covariates, ORs for continuous HAART (at least 12 months) were increased for each of the 8 bacteria analyzed

(*Fusobacterium* species, enteric rods, *P. micros*, *Campylobacter* species, *Eubacterium* species, *T. forsythia*, *P. intermedia/P. nigrescens*, *P. gingivalis*) and were significantly increased for 6 of the 8 bacteria (Table III). ORs for “new” HAART (less than 12 months) were decreased for 7 of the 8 bacteria, significantly for *P. micros*. Periodontal attachment loss was significantly associated with the presence of 6 of the 8 bacteria; for *P. intermedia/P. nigrescens* there was a significant positive association with percent gingival bleeding (OR = 2.0, CI = 1.0-4.0; *P* ≤ .05). The ORs for previous HAART were significantly decreased for yeast count. *Candida albicans* and *Candida dubliniensis* were the most prevalent yeast isolates from HIV-positive women.

Among HIV-negative specimens, the ORs associated with education level and regularity of dental visits were inconsistent across microorganisms. Periodontal attachment loss was a significant risk factor for increased *P. micros* (OR = 2.7, CI = 1.3-5.7; *P* < .05), *Eubacterium* species (OR = 9.2, CI = 3.7-23.3; *P* < .0001), and *Campylobacter* species (OR = 3.5, CI = 1.1-10.5; *P* < .05).

DISCUSSION

The present study aimed to investigate the prevalence of and the contributing factors to the presence of microorganisms in the saliva of HIV-positive women. The study examined oral and periodontal conditions as well as the medical status of HIV-positive and HIV-negative women. The bacteria studied are suspected pathogens in aggressive and chronic periodontal

diseases.⁴⁵ The study bacteria probably entered the saliva from tooth surfaces and the tongue.

In agreement with previous studies, education, dental care, and the frequency of visits to oral health care providers were positively associated with good oral health status and negatively associated with the presence of oral bacterial pathogens.⁴⁶⁻⁵⁰ Periodontal attachment loss was positively associated with the presence of all study bacteria, except for *P intermedia/P nigrescens*.

CD4 cell counts and HIV-1 RNA are commonly used as markers of immunosuppression and disease progression in HIV-infected patients. A priori, we expected to find a higher prevalence of the study microorganisms in HIV-positive than in HIV-negative women because of the immunosuppressive nature of the HIV infection. However, contrary to our initial notion, HIV seropositivity and level of immunosuppression proved not to be significant risk factors for the presence of pathogenic microorganisms in saliva, but HAART was. Our study limited the microbiological evaluation to 23 microorganisms. It is not clear if HAART directly affected the occurrence of the study microorganisms or affected another set of salivary microorganisms, thereby shifting the ecological balance in favor of the studied microorganisms. Also, the clinical consequences of the elevated occurrence of periodontopathic bacteria are not obvious. Even if it is reasonable to assume that the potential risk of destructive periodontal disease increases with rising levels of pathogenic bacteria, periodontal disease pathogenesis also involves the activation of periodontal herpesviruses,⁵¹ which takes place at a diminishing rate following HAART.⁵² Future longitudinal studies are needed to delineate the effect of HAART on the periodontal disease status. In addition to suppressing HIV replication and increasing CD4 cell counts, HAART has the potential to unmask the clinical manifestations of various nonoral opportunistic infections.⁵³⁻⁵⁷ A growing number of individuals on HAART experience unusual inflammatory responses associated with opportunistic microbial and viral pathogens as part of a new syndrome called immune restoration (reconstitution) disease (IRD).⁵⁸ IRD may involve *Mycobacterium avium* complex, *Mycobacterium tuberculosis*, cytomegalovirus, hepatitis C virus, hepatitis B virus, histoplasma capsulation, *Cryptococcus neoformans*, progressive multifocal leukoencephalopathy, and varicella-zoster virus.⁵⁹ Salivary gland enlargement, xerostomia, and oral warts also have been suggested to be consequences of IRD.^{18,20} The positive association between HAART and oral bacterial pathogens may be IRD related or constitute a pharmacotherapeutic complication of HAART in the HIV-positive women studied. Because IRD and HAART are reported to affect salivary secretion,²⁰ and, because we have documented already

the association between HAART and salivary gland hypofunction in this cohort,^{9,32} it is also possible that HAART affects salivary antibacterial proteins, which are flow dependent, leading to an elevated occurrence of selected bacterial pathogens in the saliva. Oral candidiasis was one of the most common oral lesions associated with HIV infection in WIHS before HAART initiation.² In a recent publication by our group⁶⁰ we reported a significant decrease in the incidence of oral candidiasis in WIHS after HAART initiation. In agreement with the clinical findings, we detected a significant negative association between HAART and yeast counts in saliva. Similar to previous studies of candidiasis lesions in HIV-infected individuals,^{61,62} we found the most common *Candida* species in saliva of HIV-positive women to be *C albicans* and *C dubliniensis*.

In conclusion, the present findings support the notion that HAART is an independent and significant risk factor for the occurrence of various bacterial pathogens in the saliva of HIV-positive women in the WIHS. Further studies are needed to delineate the molecular mechanisms by which HAART modulates oral microbial ecology.

The Oral Substudy of the WIHS Collaborative Study Group includes the following: New York City/Bronx Consortium, New York University (Joan Phelan, DDS, Anthony Vernillo, DDS, and Manley LaMarre, RDH); The Connie Wofsy Study Consortium of Northern California, University of California, San Francisco (Deborah Greenspan, BDS, DSc, John S. Greenspan, BDS, PhD, FRC Path, and Laurie A. Macphail, DDS); Los Angeles County/Southern California Consortium, University of Southern California, Los Angeles (Roseann Mulligan, DDS, MS, Mahvash Navazesh, DMD, Joyce Galligan, RN, DDS, Lupe Arevalo, RDH, Sharon Bautista-King, and Claudia Vargas); Chicago Consortium, University of Illinois at Chicago (Mario Alves, DDS, MS, DSc); Data Coordinating Center, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Md (Stephen J. Gange, PhD, and Yolanda Barron, MS); and NIH, The National Institute of Dental and Craniofacial Research (Maryann Redford, DDS, MPH).

The WIHS is funded by the National Institute of Allergy and Infectious Diseases, with supplemental funding from the National Cancer Institute, the National Institute of Child Health and Human Development, the National Institute on Drug Abuse, the National Institute of Dental and Craniofacial Research, the Agency for Health Care and Policy and Research, and the Centers for Disease Control and Prevention: U01-AI-35004, U01-AI-31834, U01-AI-34994, U01-AI-34989, U01-HD-32632, U01-AI-34993, U01-AI-42590.

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Reprint requests:

Dr Mahvash Navazesh, DMD
 Division of Diagnostic Sciences, Room 4320
 School of Dentistry
 University of Southern California
 925 W 34th Street
 Los Angeles, CA 90089-0641
navazesh@usc.edu

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