M. Navazesh[1]*, R. Mulligan[2],
N. Kono[3], S.K.S. Kumar[1], M. Nowicki[4],
M. Alves[5], and W.J. Mack[3]

[1]Division of Periodontology, Diagnostic Sciences and Dental Hygiene, and [2]Division of Dental Public Health and Pediatric Dentistry, 925 West 34th Street, DEN 4320, Herman Ostrow School of Dentistry of USC, University of Southern California, Los Angeles, CA, 90089–0641, USA; [3]Department of Preventive Medicine and [4]Department of Medicine, Keck School of Medicine, University of Southern California; and [5]University of Illinois at Chicago; *corresponding author, navazesh@usc.edu

Oral and Systemic Health Correlates of HIV-1 Shedding in Saliva

ABSTRACT

The relationship among oral and systemic health and HIV shedding in saliva is not well-understood. We hypothesized that oral and systemic health are associated with HIV shedding in saliva of HIV-infected women. Saliva from 127 participants enrolled in the Women's Interagency HIV Study (WIHS) was collected at repeated visits over a 5½-year study period (October 1998 through March 2004) and was evaluated for HIV-1 RNA. Demographic, lifestyle, and systemic and oral health characteristics were evaluated as possible correlates of salivary HIV-1 shedding. Multivariate models showed significantly increased risk of HIV-1 shedding in saliva as blood levels of CD4 cell counts decreased (p < 0.0001) and HIV RNA increased (p < 0.0001). Diabetes (p = 0.002) and a high proportion of gingival bleeding sites (p = 0.01) were associated with increased likelihood, while anti-retroviral therapy (p = 0.0003) and higher levels of stimulated saliva flow rates (p = 0.02) were associated with a lower likelihood of HIV-1 RNA shedding in saliva.

KEY WORDS: HIV-1 shedding, saliva, oral health.

INTRODUCTION

In 2007, about 33 million people globally were infected with HIV-1. The number of HIV infections worldwide has been stable since 2000; however, new cases continue to increase in resource-limited nations. The source of infection differs by country, and includes intercourse (vaginal or anal), injected drugs, transmission from mother to child (including breast milk), and unsafe injections or other nosocomial sources (Cohen *et al.*, 2008). The estimated incidence of HIV in the United States in 2006 was 56,300, affecting primarily blacks (45%) followed by whites (35%), Hispanics (17%), Asian/Pacific Islanders (2%), and American Indian/Alaska Natives (1%). Of these, 15,000 (27%) occurred in females (Hall *et al.*, 2008). HIV transmission in females is predominantly attributed to high-risk heterosexual contact, accounting for 80% of new infections.

Blood, semen, vaginal and cervical secretions, and breast milk are established sources of HIV transmission (Schacker *et al.*, 1996). While HIV is detected in saliva at various levels with different techniques (Liuzzi *et al.*, 1996; Shugars *et al.*, 2000; Freel *et al.*, 2003), the possibility of HIV transmission *via* saliva is remote (Scully and Porter, 2000), a finding explained by the relatively low viral load in saliva, hypotonic disruption of mononuclear leukocytes, low numbers of CD4-positive target cells, anti-HIV antibodies, and inhibition of virus by salivary carbohydrate moieties and proteins such as secretory leukocyte protease inhibitor (SLPI) (Yeung *et al.*, 1993; Shugars and Wahl, 1998; Baron *et al.*, 1999; Shugars *et al.*, 2002; Lin *et al.,* 2004; Habte *et al.,* 2006). However, the possibility of transmission of HIV and other viruses from saliva remains feasible if oral and systemic health is compromised (Greenspan *et al.*, 2000; Gandhi *et al.*, 2004).

Possible sources for viral particles, HIV virions and proviral HIV-1 DNA, include serum exudate and migration of HIV-1-containing mononuclear cells from gingival crevicular fluid (GCF) (Maticic *et al.*, 2000). Other potential sources include oral ulcerations or erosions, inflamed gingiva, and oral herpes (Campo *et al*., 2006). The impact of oral and systemic health on HIV shedding in saliva is not well-understood, and few studies have addressed this (Liuzzi *et al.*, 1996; Maticic *et al.*, 2000; Shugars *et al.*, 2000). In addition, most studies have been cross-sectional in design with limited data for a short period of time, and there is a paucity of such data in HIV-infected women.

We hypothesized that oral and systemic health factors are associated with HIV shedding in the saliva of infected women. The objective of the study was to correlate the presence of HIV-1 RNA in saliva samples from participants of

the Women's Interagency HIV Study (Barkan *et al.,* 1998; Mulligan *et al.*, 2004) with demographic, lifestyle, and medical and oral health characteristics. We believe that this is the first longitudinal study to assess the effects of oral and systemic health on HIV-1 shedding in the saliva of HIV-positive women in the United States.

MATERIALS & METHODS

Study Sample

The Women's Interagency HIV Study was established to investigate the impact of HIV infection on women in the United States. It initially enrolled 2059 HIV-seropositive and 569 demographically similar HIV-seronegative women between October 1994 and November 1995. A second recruitment enrolled 737 HIV-seropositives and 406 HIV-seronegatives between 2001 and 2002. The current substudy is based on data on HIV-positive women from both recruitments. Oral and systemic health data and saliva were collected at baseline and at follow-up evaluations every 6 mos at Los Angeles and Chicago sites. A total of 127 participants donated saliva from October 1998 through March 2004. Of these 127 women, 32 contributed data on 1 visit, 34 had 2 visits, 25 had 3 visits, 14 had 4 visits, and 22 had between 5 and 7 visits, for a total of 354 subject-visits. A complete description of the Women's Interagency HIV Study design and participants has been reported (Barkan *et al.,* 1998). The study was approved by institutional review boards, and informed consent was obtained from all participants.

Study Outcome Measures

Samples of unstimulated and chewing-stimulated whole saliva were collected under standardized conditions (Navazesh and Christensen, 1982). The stimulated saliva collection involved women chewing on a standard-sized gum base for 2 min; saliva generated was discarded. Saliva was collected for an additional 3 min and saved for further analysis.

HIV-1 RNA in saliva was measured by a commercial NASBA/NucliSens® assay (Nucleic Acid Sequence Based Amplification Assay; BioMerieux, Durham, NC, USA). The binary response variable used in data analysis was the detection of HIV-1 RNA in saliva, with the limit of detection ≥ 25 copies *per* mL. The NASBA/NucliSens® assay's software reports a quantitative result if the sample's HIV-1 RNA is within the range of 25 to approximately 5 million copies *per* mL. The performance of the assay is linear from 51 to 5.39×10^6 copies/mL HIV-1 RNA. The detection rate diminishes below 176 copies/mL (> 95% detection rate) to approximately 50% detection rate at 40 copies/mL and below (Nowicki *et al.*, 2001).

Other Data Collection

Demographic, lifestyle, and medical and oral health characteristics were evaluated as possible correlates of salivary HIV-1 shedding. Sociodemographic variables included age, race/ethnicity, and education. Lifestyle measures were alcohol use,

smoking, recreational and illicit drug use, frequency of oral sex, and HIV exposure category. Health measurements included body mass index, self-reported diabetes, hepatitis C serostatus, AIDS status, CD4+ cell count, and HIV-1 RNA in plasma. Anti-retroviral therapy use was modeled in two ways: type of anti-retroviral therapy currently used (monotherapy, combination therapy, or highly active anti-retroviral therapy), and as an indicator of highly active anti-retroviral therapy use relative to prior visits (never on, initiated, continued on, or terminated highly active anti-retroviral therapy). Oral health characteristics included the following: enlarged parotid and submandibular glands; unstimulated and stimulated salivary flow rates; hard, tender, or > 1 cm lymph nodes; plaque; gingival banding; proportion of bleeding sites; papilla with cratering; pocket depth > 4 mm; proportion of teeth with loss of attachment > 2 mm; proportion of teeth with recession; and number of decayed/missing/filled teeth, decayed/missing/filled surfaces, and decayed/filled root surfaces. We evaluated proportion rather than absolute numbers of teeth/sites affected, since the number of teeth present for evaluation varied across participants. Stimulated and unstimulated salivary flow rates were also evaluated as correlates of HIV shedding. Independent variables except for age, race, education, and HCV status were time-dependent (based on data collected at each visit). Descriptions of methodologies for oral and systemic health parameters have been published (Mulligan *et al.*, 2000, 2004; Navazesh *et al.*, 2000, 2009; Gandhi *et al.*, 2004; Greenspan *et al.*, 2004; Phelan *et al.*, 2004; Alves *et al.*, 2006).

Statistical Analysis

Univariate and multivariate analyses used logistic regression with generalized estimating equations (GEE) to account for the correlated data arising from repeated measurements from participants over multiple visits. The dependent variable was the presence of detectable HIV-1 RNA in saliva. Independent variables with univariate p-values < 0.10 were included in multivariate modeling; variables significant at p < 0.05 on multivariate modeling were retained. Due to the possible co-linearity due to the high negative correlation between CD4+ cell counts and plasma HIV-1 RNA (r = -0.54, p < 0.0001), two multivariate models were evaluated, one with CD4+ cell counts included (CD4 model), and the other with HIV-1 RNA in plasma included (RNA model). To determine if associations with salivary shedding were constant over the study follow-up, we tested interactions among all variables in the final model and study visit. To consider the quantitative nature of the HIV RNA data, we also used an ordinal logistic regression approach, categorizing the salivary HIV RNA outcome at each visit as: not detectable, 26-499, 500-4999, and ≥ 5000 copies/mL. Since HIV RNA was not detected in saliva in 60% of subject visits, and very few displayed very high RNA levels, the results did not substantially differ from the dichotomous analysis. We therefore present only the dichotomous results. A two-sided alpha level for the final model was set at 0.05. All analyses used SAS Version 9.0 software.

Table 1. Baseline Demographics and Frequency of Salivary HIV-1 Shedding (n = 127 WIHS participants)

Characteristic	
All participants, baseline salivary shedding	48/127 (38)[a]
Age (median, 35 yrs; IQR, 29-43 yrs)	
< 30	14/32 (44)
30-39	18/43 (42)
40-49	13/45 (29)
50+	3/7 (43)
Race	
White	1/5 (20)
Black	22/55 (40)
Hispanic	25/66 (38)
Other	0/1 (0)
AIDS	
No	35/98 (36)
Yes	13/29 (45)
CD4+ cells (median, 400; IQR, 246-551)	
500+	7/38 (18)
350-499	10/38 (26)
200-349	19/30 (63)
< 200	12/21 (57)
HIV-1 RNA in plasma (median, 1055; IQR, 80-13,000)	
< 1000	5/63 (8)[b]
1000-9999	16/30 (53)
10,000+	26/33 (79)
ARV therapy	
None	25/42 (60)
Mono/Combo	5/16 (31)
HAART	18/69 (26)

[a] No. with salivary shedding/total no. in demographic category (percent with salivary shedding).
[b] One participant with missing viral load at baseline.
Viral load in plasma was censored at 80 copies/mL.

RESULTS

At baseline, 48 (38%) of the 127 women had detectable HIV-1 RNA in saliva. Over all visits, 141 (40%) of 354 specimens had detectable virus (Table 1). Independent variables associated on univariate analysis with salivary shedding at p < 0.10 (Table 2) were included in multivariate modeling (Tables 3 and 4).

In the multivariate model including CD4 cell count (Table 3), the risk of HIV-1 shedding in saliva increased significantly with decreasing levels of CD4 cell counts (p < 0.0001). History of diabetes (p = 0.002) and a high proportion of gingival bleeding sites (p = 0.01) were associated with increased likelihood of HIV-1 RNA shedding, while anti-retroviral therapy (p = 0.0003) and higher stimulated salivary flow rates (p = 0.02) were associated with a lower likelihood of HIV-1 RNA shedding.

In the multivariate model including HIV-1 RNA in plasma (Table 4), the risk of HIV-1 shedding in saliva increased significantly with increasing HIV RNA (p < 0.0001). The Spearman correlations between baseline plasma and saliva HIV RNA were 0.60 (unadjusted, p < 0.0001) and 0.61 (adjusted for variables in Table 4, p < 0.001). History of diabetes (p < 0.0001), high proportion of gingival bleeding sites (p = 0.001), and decayed/filled root surfaces (p = 0.002) were associated with increased likelihood of HIV-1 RNA shedding in saliva, while anti-retroviral therapy (p = 0.002) was associated with a lower likelihood of HIV-1 RNA shedding. In both multivariate models, study visit number (*i.e.*, longer duration of follow-up) was associated with higher likelihood of HIV shedding in saliva (p < 0.0001), indicating increasing prevalence of HIV shedding over follow-up (baseline prevalence = 38%, median prevalence visits 1-3 = 30%; median prevalence visits 4-6 = 44%; median prevalence visits 7-9 = 62%).

DISCUSSION

Forty-eight (38%) of the 127 women had detectable HIV-1 RNA in saliva in all study visits, whereas, overall, 141 (40%) of 354 specimens had a detectable salivary viral load. This was comparable with results from an earlier study, where 42% of participants had HIV-1 RNA shedding in saliva (Shugars *et al.*, 2000). However, this is not comparable with results from other studies that reported very high (96%) (Liuzzi *et al.*, 1996) or very low (1%) (Barr *et al.*, 1992) prevalence of salivary HIV-1 RNA shedding. The use of different collection methods and/or molecular techniques for measuring salivary viral shedding may explain these differences.

Higher HIV-1 RNA viral load in plasma was strongly associated with a higher likelihood of salivary viral shedding. This was noted in earlier studies (Liuzzi *et al.*, 1996; Shugars *et al.*, 2000) and reinforces the idea that saliva may be a useful, non-invasive source for estimating plasma viral load (Shugars *et al.*, 2000). In addition, decreased CD4+ cell count and positive AIDS status (in univariate models only) increased the risk of HIV-1 shedding in saliva, suggesting an effect of immunosuppression on salivary viral shedding.

The advent of highly active anti-retroviral therapy has significantly reduced HIV viral load systemically in plasma and other body fluids, including saliva (Shugars *et al.*, 2000). This was evident in the Women's Interagency HIV Study, where participants on highly active anti-retroviral therapy were less likely to have detectable HIV-1 RNA in saliva than participants not on anti-retroviral therapy.

Diabetes increased the likelihood of salivary HIV-1 shedding. Alteration in saliva composition is noted in diabetics with otherwise normal oral and systemic health. The mechanism by which diabetes increases HIV-1 shedding in saliva is unknown and deserves further investigation (Yavuzyilmaz *et al.*, 1996).

We previously reported the association among salivary gland hypofunction, HIV-1 infection, and highly active anti-retroviral therapy in the Women's Interagency HIV Study (Navazesh *et al.*, 2003, 2009). The current investigation revealed a significant association between lower volume of chewing-stimulated saliva and higher risk for HIV-1 shedding in saliva. This could imply that endogenous viral inhibitors suppress HIV-1 shedding in saliva.

Interestingly, we observed an increase in prevalence of HIV-1 shedding in saliva with longer follow-up (higher subject visits). The mean plasma viral loads were elevated beyond the baseline level in later study visits (beyond visit 5) in some participants, which could increase the HIV-1 shedding in saliva

Table 2. Univariate Logistic Regression of HIV Shedding in Saliva

Variable	Salivary HIV-1 Shedding	OR (95% CI)	P-value
Alcohol use in last 6 months			
No	71/207 (34)[a]	1.00	
Yes	68/143 (48)	1.57 (0.97-2.54)	0.07
Drug use in last 6 months (crack, cocaine, heroin, IDU)			
No	117/318 (37)	1.00	
Yes	22/34 (65)	2.87 (1.08-7.63)	0.03
Diabetes			
No	131/338 (39)	1.00	
Yes	9/15 (60)	2.75 (0.95-7.99)	0.06
AIDS			
No	90/247 (36)	1.00	
Yes	51/107 (48)	1.59 (0.95-2.68)	0.08
ARV therapy at study visit			
None	57/96 (59)	1.00	0.0002[b]
Mono/Combo	17/45 (38)	0.32 (0.14-0.75)	
HAART	66/212 (31)	0.28 (0.16-0.48)	
CD4+ cell count			
500+	37/139 (27)	1.00	< 0.0001[b]
350-499	27/84 (32)	1.38 (0.75-2.53)	
200-349	37/65 (57)	3.56 (1.76-7.20)	
< 200	39/62 (63)	4.72 (2.46-9.06)	
HIV-1 RNA in plasma			
< 1000	39/202 (19)	1.00	< 0.0001[b]
1000-9999	34/67 (51)	4.16 (2.15-8.08)	
10,000+	67/81 (83)	19.6 (10.3-37.3)	
Plaque			
No or probe only	17/57 (30)	1.00	
Visible	111/273 (41)	1.57 (0.93-2.68)	0.09
Average gingival banding			
None	111/303 (37)	1.00	
Present	17/27 (63)	2.22 (0.97-5.10)	0.06
Decayed/filled root surfaces			
None	103/278 (37)	1.00	
Present	29/57 (51)	1.64 (0.91-2.96)	0.10
Stimulated salivary flow rate, mL/min[c]			
Positive for shedding[d]	0.67 (0.67)[d]	0.67 (0.46-0.98)	0.04
No shedding	0.83 (0.75)		
Proportion of teeth with plaque visible[c]			
Positive for shedding[d]	0.25 (0.40)[d]	2.08 (0.93-4.66)	0.08
No shedding	0.19 (0.39)		
Proportion of bleeding sites[c]			
Positive for shedding[d]	0.20 (0.30)[d]	4.28 (1.59-11.5)	0.004
No shedding	0.14 (0.24)		
Visit[c]		1.16 (1.07-1.25)	0.0003[b]

Table includes independent variables associated with salivary shedding at p ≤ 0.10. Parameter estimates and significance tests are from logistic regression with generalized estimating equations.
[a] No. of visits with salivary shedding/total visits (percent).
[b] P-value for trend.
[c] ORs for continuous variables are based on a one-unit change in the predictor; for study visit, one-unit change = 6 months.
[d] Median (IQR) by salivary shedding status.

(results not shown here). We speculate that this could be due to non-compliance with HIV medication intake or development of resistance to medications.

We observed a significant association between the proportion of bleeding sites and the risk of HIV-1 shedding in saliva. Similarly, increased linear gingival banding and visible plaque (only in the univariate model) were associated with increased HIV shedding that was similar to a previous observation (Maticic *et al.*, 2000; Shugars *et al.*, 2000). Inflammatory conditions such as linear gingival banding or erythema may increase viral shedding through ulcerated surfaces or *via* gingival crevicular fluid (Shugars *et al.*, 2000). However, these findings conflict with earlier reports indicating no significant association between infectious HIV-1 in whole saliva and periodontal disease (Barr

Table 3. Multivariate Logistic Regression (CD4 model)[a]

Variable	OR (95% CI)	P-value
Visit[b]	1.23 (1.11-1.37)	< 0.0001
Diabetes		
No	1.00	
Yes	3.93 (1.64-9.38)	0.002
ARV therapy		
None	1.00	0.0003[c]
Mono/Combo	0.35 (0.12-1.01)	
HAART	0.23 (0.12-0.46)	
CD4+ cell count		
500+	1.00	< 0.0001[c]
350-499	1.58 (0.78-3.21)	
200-349	3.78 (1.65-8.58)	
< 200	4.90 (2.17-11.1)	
Stimulated salivary flow rate, mL/min	0.57 (0.36-0.91)	0.02
Proportion of bleeding sites	1.42 (1.10-1.84)[d]	0.01

[a] Logistic regression with generalized estimating equations (319 visits, 119 subjects), using exchangeable correlation.
[b] OR *per* one unit change in study visit = 6 months.
[c] P-value for trend.
[d] OR based on a one standard deviation change in the independent variable.

Table 4. Multivariate Logistic Regression (RNA model)[a]

Variable	OR (95% CI)	P-value
Visit[b]	1.37 (1.21-1.54)	< 0.0001
Diabetes		
No	1.00	
Yes	5.86 (2.45-14.0)	< 0.0001
ARV therapy		
None	1.00	0.002[c]
Mono/Combo	0.67 (0.22-2.10)	
HAART	0.30 (0.15-0.63)	
HIV-1 RNA in plasma		
< 1000	1.00	< 0.0001[c]
1000-9999	5.47 (2.61-11.5)	
10,000+	43.4 (18.8-100)	
Decayed/filled root surfaces		
None	1.00	
Present	2.84 (1.45-5.56)	0.002
Proportion of bleeding sites	1.65 (1.22-2.24)[d]	0.001

[a] Logistic regression with generalized estimating equations (321 visits, 120 participants) using exchangeable correlation.
[b] OR *per* one-unit change in study visit = 6 months.
[c] P-value for trend.
[d] OR based on a one standard deviation change in the independent variable.

et al., 1996). These differences could be attributed to differences in populations and different sensitivities of the HIV assays. Among the other dental variables, the presence of decayed/filled root surfaces increased the risk of HIV-1 shedding in saliva.

Limitations of this study include the fact that some independent variables were based on participant self-report and hence could be subject to higher measurement error. Despite the fact that multiple systemic and oral health measures were obtained from the same cohort, the comprehensive nature of measures examined, as well as the longitudinal study design, make it more challenging for objective assessments to be obtained for all the variables. Given the large number of associations tested, it is possible that some of our findings may have arisen by chance; however, the highly significant associations noted in our multivariate models make this possibility unlikely. Nonetheless, these findings should be replicated in an independent sample.

To our knowledge, this is the first comprehensive evaluation of HIV-1 shedding in saliva of HIV-infected women. We observed specific oral and systemic health parameters to be associated with HIV-1 RNA shedding in saliva of HIV-infected women in the Women's Interagency HIV Study.

ACKNOWLEDGMENTS

REFERENCES

Alves M, Mulligan R, Passaro D, Gawell S, Navazesh M, Phelan J*, et al.* (2006). Longitudinal evaluation of loss of attachment in HIV-infected women compared to HIV-uninfected women. *J Periodontol* 77: 773-779.

Barkan SE, Melnick SL, Preston-Martin S, Weber K, Kalish LA, Miotti P*, et al.* (1998). The Women's Interagency HIV Study. WIHS Collaborative Study Group. *Epidemiology* 9:117-125.

Baron S, Poast J, Cloyd MW (1999). Why is HIV rarely transmitted by oral secretions? Saliva can disrupt orally shed, infected leukocytes. *Arch Intern Med* 159:303-310.

Barr CE, Miller LK, Lopez MR, Croxson TS, Schwartz SA, Denman H*, et al.* (1992). Recovery of infectious HIV-1 from whole saliva. *J Am Dent Assoc* 123:36-37, 39-48.

Barr CE, Qureshi MN, Qiu Z, Kaim J, Zhang W (1996). Oral HIV-I recovery in the presence of periodontal disease. *Oral Dis* 2:198-201.

Campo J, Perea MA, del Romero J, Cano J, Hernando V, Bascones A (2006). Oral transmission of HIV, reality or fiction? An update. *Oral Dis* 12:219-228.

Cohen MS, Hellmann N, Levy JA, DeCock K, Lange J (2008). The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J Clin Invest* 118:1244-1254.

Freel SA, Fiscus SA, Pilcher CD, Menezes P, Giner J, Patrick E, *et al*. (2003). Envelope diversity, coreceptor usage and syncytium-inducing phenotype of HIV-1 variants in saliva and blood during primary infection. *AIDS* 17:2025-2033.

Gandhi M, Koelle DM, Ameli N, Bacchetti P, Greenspan JS, Navazesh M, *et al*. (2004). Prevalence of human herpesvirus-8 salivary shedding in HIV increases with CD4 count. *J Dent Res* 83:639-643.

Greenspan D, Komaroff E, Redford M, Phelan JA, Navazesh M, Alves ME, *et al*. (2000). Oral mucosal lesions and HIV viral load in the Women's Interagency HIV Study (WIHS). *J Acquir Immune Defic Syndr* 25:44-50.

Greenspan D, Gange SJ, Phelan JA, Navazesh M, Alves ME, MacPhail LA, *et al*. (2004). Incidence of oral lesions in HIV-1-infected women: reduction with HAART. *J Dent Res* 83:145-150.

Habte HH, Mall AS, de Beer C, Lotz ZE, Kahn D (2006). The role of crude human saliva and purified salivary Muc5b and Muc7 mucins in the inhibition of Human Immunodeficiency Virus type 1 in an inhibition assay. *Virol J* 3:99.

Hall HI, Song R, Rhodes P, Prejean J, An Q, Lee LM, *et al*. (2008). Estimation of HIV incidence in the United States. *JAMA* 300:520-529.

Lin AL, Johnson DA, Stephan KT, Yeh CK (2004). Salivary secretory leukocyte protease inhibitor increases in HIV infection. *J Oral Pathol Med* 33:410-416.

Liuzzi G, Chirianni A, Clementi M, Bagnarelli P, Valenza A, Cataldo PT, *et al*. (1996). Analysis of HIV-1 load in blood, semen and saliva: evidence for different viral compartments in a cross-sectional and longitudinal study. *AIDS* 10:F51-F56.

Maticic M, Poljak M, Kramar B, Tomazic J, Vidmar L, Zakotnik B, *et al*. (2000). Proviral HIV-1 DNA in gingival crevicular fluid of HIV-1-infected patients in various stages of HIV disease. *J Dent Res* 79:1496-1501.

Mulligan R, Navazesh M, Komaroff E, Greenspan D, Redford M, Alves M, *et al*. (2000). Salivary gland disease in Human Immunodeficiency Virus-positive women from the WIHS Study. Women's Interagency HIV Study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 89:702-709.

Mulligan R, Phelan JA, Brunelle J, Redford M, Pogoda JM, Nelson E, *et al*. (2004). Baseline characteristics of participants in the oral health component of the Women's Interagency HIV Study. *Community Dent Oral Epidemiol* 32:86-98.

Navazesh M, Christensen CM (1982). A comparison of whole mouth resting and stimulated salivary measurement procedures. *J Dent Res* 61:1158-1162.

Navazesh M, Mulligan R, Komaroff E, Redford M, Greenspan D, Phelan J (2000). The prevalence of xerostomia and salivary gland hypofunction in a cohort of HIV-positive and at-risk women. *J Dent Res* 79:1502-1507.

Navazesh M, Mulligan R, Barron Y, Redford M, Greenspan D, Alves M, *et al*. (2003). A 4-year longitudinal evaluation of xerostomia and salivary gland hypofunction in the Women's Interagency HIV Study participants. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 95:693-698.

Navazesh M, Mulligan R, Karim R, Mack WJ, Ram S, Seirawan H, *et al*. (2009). Effect of HAART on salivary gland function in the Women's Interagency HIV Study (WIHS). *Oral Dis* 15:52-60.

Nowicki MJ, Benning L, Bremer JW, Meyer WA 3rd, Hanson C, Brambilla D, *et al*. (2001). Longitudinal variability of Human Immunodeficiency Virus type 1 RNA viral load measurements by nucleic acid sequence-based amplification and Nuclisens assays in a large multicenter study. *J Clin Microbiol* 39:3760-3763.

Phelan JA, Mulligan R, Nelson E, Brunelle J, Alves ME, Navazesh M, *et al*. (2004). Dental caries in HIV-seropositive women. *J Dent Res* 83:869-873.

Schacker T, Collier AC, Hughes J, Shea T, Corey L (1996). Clinical and epidemiologic features of primary HIV infection. *Ann Intern Med* 125:257-264.

Scully C, Porter S (2000). HIV topic update: oro-genital transmission of HIV. *Oral Dis* 6:92-98.

Shugars DC, Wahl SM (1998). The role of the oral environment in HIV-1 transmission. *J Am Dent Assoc* 129:851-858.

Shugars DC, Slade GD, Patton LL, Fiscus SA (2000). Oral and systemic factors associated with increased levels of Human Immunodeficiency Virus type 1 RNA in saliva. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 89:432-440.

Shugars DC, Sweet SP, Malamud D, Kazmi SH, Page-Shafer K, Challacombe SJ (2002). Saliva and inhibition of HIV-1 infection: molecular mechanisms. *Oral Dis* 8(Suppl 2):169-175.

Yavuzyilmaz E, Yumak O, Akdoganli T, Yamalik N, Özer N, Ersoy F, *et al*. (1996). The alterations of whole saliva constituents in patients with diabetes mellitus. *Aust Dent J* 41:193-197.

Yeung SC, Kazazi F, Randle CG, Howard RC, Rizvi N, Downie JC, *et al*. (1993). Patients infected with Human Immunodeficiency Virus type 1 have low levels of virus in saliva even in the presence of periodontal disease. *J Infect Dis* 167:803-809.