Oral Diseases (2009) 15, 52–60. doi:10.1111/j.1601-0825.2008.01456.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Effect of HAART on salivary gland function in the Women's Interagency HIV Study (WIHS)

M Navazesh, R Mulligan, R Karim, WJ Mack, S Ram, H Seirawan, J Greenspan, D Greenspan, J Phelan, M Alves, The Oral Substudy of the WIHS Collaborative Study Group*

OBJECTIVE: To determine the impact of highly active antiretroviral therapy (HAART) on salivary gland function in human immunodeficiency virus (HIV) positive women from the Women's Interagency HIV Study (WIHS).
DESIGN: Longitudinal cohort study.
SUBJECTS AND METHODS: A total of 668 HIV positive women from the WIHS cohort with an initial and at least one follow-up oral sub-study visit contributed 5358 visits. Salivary gland function was assessed based on a dry mouth questionnaire, whole unstimulated and stimulated salivary flow rates, salivary gland enlargement or tenderness and lack of saliva on palpation of the major salivary glands.
MAIN OUTCOME MEASURES: Changes in unstimulated and stimulated flow rates at any given visit from that of the immediate prior visit (continuous variables). The development of self-reported dry mouth (present/absent), enlargement or tenderness of salivary glands (present/absent), and absence of secretion on palpation of the salivary glands were binary outcomes (yes/no).
RESULTS: Protease Inhibitor (PI) based HAART was a significant risk factor for developing decreased unstimulated ($P = 0.01$) and stimulated ($P = 0.0004$) salivary flow rates as well as salivary gland enlargement ($P = 0.006$) as compared with non-PI based HAART.
CONCLUSIONS: PI-based HAART therapy is a significant risk factor for developing reduced salivary flow rates and salivary gland enlargement in HIV positive patients.
Oral Diseases (2009) **15**, 52–60

Keywords: HIV; antiretroviral therapy; HAART; salivary gland function; salivary gland enlargement; PI based HAART

Introduction

Saliva plays an important role in maintaining the health of the oral cavity. Xerostomia (subjective complaint of dry mouth) and salivary gland hypofunction (objective evidence of reduced salivary output) have often been associated with the human immunodeficiency virus (HIV) infection (Navazesh *et al*, 2000). The prevalence of xerostomia and salivary gland hypofunction has been reported to be 2–10% in HIV infected patients (Laskaris *et al*, 1992; Schiodt, 1997; Ramos-Gomez *et al*, 1999; Kozinetz *et al*, 2000; Navazesh *et al*, 2000). Numerous studies have reported an alteration of salivary gland function and composition in HIV patients in both early and advanced stages of infection (Yeh *et al*, 1988; Navazesh *et al*, 2000, 2003; Lin *et al*, 2003).

In recent years, highly active antiretroviral therapy (HAART) has become the management modality of choice in the management of HIV infection. HAART, consisting of two nucleotide analogues and a protease inhibitor (PI), is a currently recommended regimen for suppressing HIV replication and for restoring or preserving host immunity (Lin *et al*, 2006). Xerostomia (dry mouth) (Scully and Diz Dios, 2001) and lipodystrophic changes of the salivary glands (Olive *et al*, 1998) have been reported as potential adverse effects of PI therapy. HAART therapy has changed the quality and length of life for individuals with HIV infection. Recent studies have shown a decrease in the prevalence of the salivary gland disorder diffuse infiltrative lymphocytosis syndrome (DILS) (also referred to as Sjögren–like syndrome or SLS by Panayiotakopoulous

Correspondence: M Navazesh, 925 West 34th Street, DEN 4320, USC School of Dentistry, Los Angeles, CA 90089-0641, USA. Tel: +1 213 740 1418, Fax: +1 213 821 5278, E-mail: navazesh@usc.edu
*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, Wendy Mack, PhD, Roksana Karim, PhD, Saravanan Ram, BDS, MDS, Hazem Seirawan, PhD, Sharon Bautista-King, and Claudia Vargas); Chicago Consortium, University of Illinois at Chicago (Mario Alves, DDS, MS, DSc); Data Coordinating Center, John 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).
Received 5 February 2008; accepted 06 May 2008

et al) that are unique to HIV-infected individuals (Panayiotakopoulos *et al*, 2003; Basu *et al*, 2006). Recently, Lin *et al* (Lin *et al*, 2006) conducted a longitudinal study to assess the effects of HAART therapy on salivary gland flow rates and composition among 39 HIV-negative and 147 HIV-positive patients (69 on HAART therapy and 78 not treated with HAART). There was a significant decrease in the salivary flow rates of the HIV positive group as compared to the control group, but the flow rates of patients on HAART was not significantly different from those not on HAART. All the participants in this study were men.

We hypothesized that HAART has no impact on salivary gland function in HIV infected women. To test this hypothesis we investigated the effect of HAART on salivary gland function in a large cohort of HIV infected women who were participating in the Women's Interagency HIV Study (WIHS). To our knowledge, this is the first longitudinal and comprehensive evaluation of HAART and specifically PI-based HAART, in a culturally diverse and large population of HIV-infected women.

Materials and methods

Description of the WIHS cohort

The WIHS was established in August 1993 to investigate the impact of HIV infection on women in the United States. Details of the WIHS design and study population have been published previously (Barkan *et al*, 1998). Briefly, WIHS is an ongoing multi-centre cohort study that initially enrolled 2059 HIV-seropositive and 569 demographically similar HIV-seronegative women since 1994. The current sub-study is based on data on HIV positive women from baseline visit 1 (occurring between 1 October 1994 and 31 March 1995) through visit 18 (occurring between 1 April 2003 and 1 September 2003) collected at baseline and at 6-month follow-up evaluations over 10 years at four examination sites in Chicago, Los Angeles, New York, and San Francisco. All study participants provided informed consent to the research protocols that had been approved by the appropriate institutional review boards and the Health Sciences Institutional Review Board (HSIRB) and Ethics Committee of the University of Southern California. The research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version, 2002).

A total of 668 HIV positive women with an initial and at least one follow-up oral sub-study visit contributed 5358 women-visits to the analysis. The total number of visits for a subject ranged from 2 to 17, with a median of 7. Study subjects received medical and oral as well as salivary gland examinations at baseline and every 6 months (Mulligan *et al*, 2004). At each visit, an extensive medical interview (including patients' self-report of medication use), physical examination (including a comprehensive head and neck examination), and laboratory (serological and hematological) evaluations were performed by a trained examiner. All examiners received standardized training in performing the study protocols. The inspection and palpation of the salivary glands and saliva collection techniques are described in detail elsewhere (Navazesh *et al*, 2000). Salivary flow rates were determined in ml per min and reflect values of whole saliva.

Assessment of salivary gland function

Salivary gland function was assessed based on the following measures: (1) the participant's responses to a dry mouth questionnaire; (2) evidence of enlargement, tenderness and lack of saliva secretion upon visual inspection and palpation of the major salivary glands; and (3) the unstimulated and chewing-stimulated whole saliva flow rates. A positive self-report of dry mouth included positive responses on one of the following questionnaire items: (1) Does the amount of saliva in your mouth seem to be too little? (2) Do you have any difficulties swallowing? (3) Does your mouth feel dry when eating a meal? (4) Do you sip liquids to aid in swallowing dry foods?

Definition of HAART

At each visit, participants were asked about their current anti-retroviral therapy (ART) use as well as ART usage in the past 6 months. The definition of HAART regimens was guided by the 2000 versions of the U.S. Department of Health and Human Services (US Department of Health and Human Services, 2002) and the International AIDS Society-USA Panel antiretroviral guidelines (Yeni *et al*, 2002). Women were considered to be on HAART if they were taking: (a) two or more nucleoside reverse transcriptase inhibitors (NRTI) with at least one PI or non-nucleoside reverse transcriptase inhibitor (NNRTI); (b) one NRTI in combination with at least one PI and at least one NNRTI; (c) a regimen containing ritonavir and saquinavir in combination with one NRTI and no NNRTIs; or (d) an abacavir-containing regimen of three or more NRTI in the absence of both PI and NNRTI. Monotherapy included either one NRTI (95%) or only PI (4%) or only NNRTI (1%). All other regimens were defined as combination therapy. At each study visit, PI-based HAART users were defined as women taking HAART that included a PI. Non PI-based HAART users were defined as women taking HAART without PI at any given visit.

Measurement of HIV RNA levels

HIV-1 RNA levels were measured with commercial NASBA/NucliSens assays (Nucleic Acid Sequence Based Amplification Assay; BioMerieux, Durham, NC, USA) combined with 1 ml of specimen. According to the manufacturer's recommendation, specimens with ECL (electrochemiluminescently labeled probes) signal strength of at least 250 Units and >25 HIV-1 copies ml^{-1} reported by the manufacturer software were considered to be reactive.

Statistical analysis

We evaluated the following measures of salivary gland function:

(1) change in the unstimulated and stimulated flow rates at any given visit from that of the immediately prior visit (continuous variables);
(2) development of self-reported dry mouth in response to the dry mouth questionnaire items (per previous description, a positive response on any item);
(3) development of enlargement or tenderness of the salivary glands (present or absent); and
(4) development of lack of secretion on palpation of the salivary glands (yes/no).

Each of these measures of salivary gland function was evaluated in relation to HAART use and changes in HAART use. HAART use was defined as 'current nonuser' (not on HAART at current visit and the prior visit), 'initiators' (not on HAART at prior visit but started HAART some time between the previous visit and the current visit), 'stoppers' (used HAART in the prior visit but not at the current visit), and 'maintainers' (used HAART both at the current and prior visits). These HAART categories were defined for any HAART use. Subsequent models evaluated PI-based and non-PI based HAART separately, using the same definitions of initiation, maintenance and cessation. Impact of any PI, irrespective of HAART regimen or combination therapy regimen, was also examined in relation to development of the salivary gland outcomes.

Other factors tested for an association with salivary gland outcomes were: age (<35, 35–44, $45+$), race (white, black, Hispanic, and others), HIV RNA viral load (<5000, 5000–9999, 10 000–49 999, 50 000–99 999, 100 000+ copies ml^{-1}) (Levine *et al*, 2006), CD4 cell count (>500, 350–499, 200–349, <200 cells μl^{-1}), the presence of an AIDS-defining illness, depressed mood [measured by the Centre for Epidemiologic Studies Depression (CESD) scale, modeled as a continuous variable], hepatitis C (HCV) infection, and use of antidepressant, anti-diabetic and anti-hypertensive medications. Except for baseline age, race, and HCV status, all other exposure variables were time dependent (i.e., modeled as visit-specific measures).

Linear (for continuous outcomes) or logistic (for binary outcomes) regression models were used with generalized estimating equations (GEE) to analyze the data accounting for the within subject correlation of the repeated measures of salivary gland outcome. An exchangeable correlation matrix was assumed among the repeated measures of salivary gland outcomes. Separate models were tested for each of the salivary gland outcome measures. Changes in unstimulated and stimulated salivary flow rates at any given visit from the salivary flow rate of the previous visit were modeled as continuous outcome variables. Therefore, linear regression models with GEE were used for this analysis. Women who were unable to contribute unstimulated saliva because of the scantiness or complete nonexistence of flow did not contribute to the calculation and analysis of salivary flow rate change in the subsequent visit. In total 651 of 3136 women-visits (21%) were excluded for this reason.

Binary salivary gland outcomes, including a positive response to the dry mouth questionnaire, enlargement and tenderness of the salivary glands, and no secretion on palpation of the salivary glands, were analyzed using logistic regression models with GEE. To evaluate factors related to the development of any of these conditions, only women who were negative on the particular outcome in the previous visit contributed to the analysis at any given visit. At any visit, we compared women who did or did not develop the outcome, among those who were 'at risk' to develop the outcome. Both univariate and multivariate analyses were performed. Correlates with a P-value <0.15 in the univariate analysis were tested in the multivariate models. All analyses used statistical analysis software (SAS) version 9.1 (Cary, NC, USA).

Results

The baseline characteristics of the study participants are summarized in Table 1. The median (range) age of the study participants was 37 (17–60) years. The majority of the participants were black (54%); other ethnic groups included Hispanic (29%), white (14%), and other ethnic backgrounds (3%). At baseline, 17% of the women had an AIDS-defining illness and 46% were infected with hepatitis C virus.

Associations of HAART with longitudinal changes in salivary gland outcomes (Table 2)

In a multivariable model adjusted for age, viral load and CD4 count, AIDS defining illness and depression score, longitudinal change in the unstimulated salivary flow

Table 1 Baseline characteristics of the WIHS oral sub-study participants ($n = 668$)

Age (years)	*37(17–60)*[a]
Race (%)	
White	14
Black	54
Hispanic	29
Others	3
HIV RNA (copies/ml)	9300 (80–9 300 000)
CD4 cell count (cells/μl)	392 (0–3838)
CESD[b]	18 (0–58)
AIDS outcomes (%)	17
HCV positive (%)	46
Stimulated salivary flow rate (ml/min)	0.83 (0–31)
Unstimulated salivary flow rate (ml/min)	0.20 (0–2.5)
Yes response to 'difficulty swallowing or dry mouth when eating and or sipping liquids' (%)	49
Yes response to 'too little saliva' question (%)	22
Salivary gland enlargement (%)	5
Salivary gland tenderness (%)	6
Absence of saliva on palpation (%)	32

[a]Continuous variables are expressed in median (range).
[b]CESD – Centre for Epidemiologic Studies Depression Scale.

Table 2 Impact of HAART (non-PI or PI-based) on salivary gland function

	All HAART		*Non PI-based HAART*		*PI-based HAART*	
	Estimate (SE)[a]	P-*value*	*Estimate (SE)*[a]	P-*value*	*Estimate (SE)*[a]	P-*value*
Changes in stimulated flow rate (ml/min)						
HAART use						
No HAART[b]	Referent		Referent		Referent	
Ended HAART	−0.04 (0.05)	0.40	−0.10 (0.07)	0.14	−0.02 (0.04)	0.67
Initiated HAART	−0.02 (0.04)	0.60	−0.08 (0.06)	0.16	0.02 (0.04)	0.67
Maintained	−0.14 (0.03)	<0.0001	−0.05 (0.05)	0.23	−0.11 (0.03)	0.0004
Changes in unstimulated flow rate (ml/min)						
No HAART	Referent		Referent		Referent	
Ended HAART	0.02 (0.03)	0.40	0.02 (0.03)	0.52	−0.0005 (0.03)	0.87
Initiated HAART	0.004 (0.01)	0.81	0.34 (0.03)	0.29	−0.0005 (0.02)	0.80
Maintained	−0.02 (0.01)	0.17	0.01 (0.03)	0.60	−0.04 (0.02)	0.01
	OR (95% CI)[b]	P-*value*	*OR (95% CI)*[b]	P-*value*	*OR (95% CI)*[b]	P-*value*
Complaint of too little saliva						
HAART use						
No HAART	Referent		Referent		Referent	
Ended HAART	0.82 (0.50–1.34)	0.42	0.55 (0.23–1.29)	0.17	1.05 (0.65–1.69)	0.84
Initiated HAART	0.80 (0.56–1.12)	0.19	1.18 (0.72–1.92)	0.55	0.80 (0.54–1.18)	0.26
Maintained	0.73 (0.55–0.97)	0.03	0.67 (0.41–1.10)	0.64	0.79 (0.59–1.08)	0.14
Enlargement of the salivary gland						
No HAART	Referent		Referent		Referent	
Ended HAART	2.14 (1.04–4.44)	0.04	2.43 (0.94–6.27)	0.07	1.22 (0.57–2.58)	0.61
Initiated HAART	1.19 (0.64–2.19)	0.58	0.78 (0.30–2.05)	0.62	1.05 (0.56–1.93)	0.89
Maintained	2.20 (1.44–3.38)	0.003	1.56 (0.81–2.99)	0.18	1.82 (1.18–2.80)	0.006

[a]Outcome variable of the model was change in the salivary flow rate from one visit to the next as a continuous variable. β-estimates (SE) from generalized estimating equations represent mean differences from the referent group (no HAART) on the outcome variable.
[b]Odds Ratio (95% Confidence Interval) for the binary outcome variables from generalized estimating equations.
Adjustment variables were included based on univariate associations and were different for each outcome model as listed below:
Stimulated salivary flow rate: age, race, viral load, CD4 count, depression score, hepatitis C status, medication use for diabetes, hypertension and depression.
Unstimulated salivary flow rate: age, viral load and CD4 count, AIDS defining illness and depression score.
Complaint of too little saliva: age, viral load, CD4 count, depression score, hepatitis C infection, AIDS defining illness, and medication for diabetes and depression.
Enlargement of the salivary glands: race, AIDS defining illness and medication for depression.

rate was not associated with HAART use. Multivariate regression analysis adjusted for age, race, viral load, CD4 count, depression score, hepatitis C status, medication use for diabetes, hypertension and depression, showed that the stimulated salivary flow rate significantly decreased among women maintaining HAART, compared to women who did not use HAART at the current and prior visits ($P < 0.0001$; Table 2). Subsequent analysis of the effects of PI and non-PI based HAART use on the salivary flow rates showed that only PI based HAART was significantly associated with a decrease in both the unstimulated ($P = 0.01$) and stimulated salivary flow rates ($P = 0.0004$) in those women maintaining HAART. Women who had no measurable unstimulated salivary flow (21%) were excluded from the analysis reported above. In a sensitivity analysis, we repeated these analyses of changes in the unstimulated salivary flow rate including all women visits. The results were similar i.e., there was a significant reduction in the unstimulated salivary flow rate among women who were on PI-based HAART for two sequential WIHS visits (data not shown).

Women who maintained use of HAART over two study visits had a reduced risk of developing a complaint of too little saliva (subjective report of dryness) (OR = 0.73; CI = 0.55–0.97; $P = 0.03$) compared with women not using HAART (Table 2; result adjusted for age, viral load, CD4 count, depression, hepatitis C infection, AIDS defining illness, and medication for diabetes and depression). This trend of reduced risk was equally evident in women maintaining PI-based and non-PI based HAART, but was not significant due to reduced sample size.

Compared with women who were not on HAART, those who used HAART in the past visit but had stopped at the current visit (OR = 2.14; CI = 1.04–4.44; $P = 0.04$) and those who maintained HAART for at least two consecutive visits (OR = 2.20; CI = 1.44–3.38; $P = 0.003$) were at greater risk for developing enlarged salivary glands (adjusted for race, AIDS defining illness and medication for depression). The elevated risk associated with maintaining HAART was statistically significant in women who maintained use of PI-based HAART (OR = 1.82; CI = 1.18–2.80; $P = 0.006$), but was not significant in women who maintained use of non-PI based HAART (Table 2). HAART use was not associated with the risk of developing tender salivary glands or lack of secretion on palpation.

Table 3 Impact of PI use (irrespective of HAART use) on stimulated salivary flow rate

Model variables	*β-estimate (SE)*	P-*value*
PI use		
No	Referent	
Yes	−0.06 (0.03)	0.02
Age (years)	0.004 (0.003)	0.15
Race		
White	Referent	
Black	0.04 (0.07)	0.50
Hispanic	−0.05 (0.07)	0.43
Other	−0.17 (0.09)	0.05
HIV RNA (copies/ml)		
< 5000	Referent	
5000–9999	0.02 (0.04)	0.56
10 000–49 999	−0.05 (0.03)	0.13
50 000–99 999	−0.05 (0.06)	0.43
≥100 000	−0.09 0.05)	0.10
CD4 count/μl		
> 500	Referent	
350–500	0.01 (0.03)	0.64
200–349	−0.02 (0.04)	0.57
< 200	0.004 (0.05)	0.94
Hepatitis C infection		
No	Referent	
Yes	−0.11 (0.05)	0.03
Diabetes mellitus		
No	Referent	
Yes	−0.25 (0.06)	< 0.0001
Antihypertensive medication		
No	Referent	
Yes	−0.16 (0.07)	0.02
Antidepressant medication		
No	Referent	
Yes	−0.11 (0.05)	0.03
Changes in the stimulated salivary flow rate sin immediately prior visit (ml/min)	−0.85 (0.07)	< 0.0001

Outcome variable of the model was change in the stimulated salivary flow rate from one visit to the next as a continuous variable. β-estimates (SE) from generalized estimating equations represent mean differences from the referent group on the outcome variable.

Associations of PI use (irrespective of HAART) with longitudinal changes in salivary gland outcomes

PI use was significantly associated with reduced stimulated salivary flow rate ($P = 0.02$) (Table 3). Other salivary gland outcomes including changes in unstimulated salivary flow rate, complaint of too little saliva, enlargement of the salivary gland, tenderness of the salivary gland, and lack of secretion on palpation of the salivary gland were not significantly associated with PI use.

Associations of HIV RNA levels and CD4 counts with changes in unstimulated salivary flow

HIV RNA levels between 10 000–49 999 copies ml^{-1} and more than or equal to 100 000 were significantly associated with a reduction in the unstimulated salivary flow rate compared with that of < 5000 copies ml^{-1}. CD4 counts in the range of 200–349 counts μl^{-1} were also significantly associated with reduced unstimulated salivary flow rate compared with CD4 > 500 counts μl^{-1} (Table 4).

Table 4 Changes in the unstimulated salivary flow rate in relation to HIV RNA and CD4 count

	Total sample	
HIV RNA (copies/ml)	Estimate[a]	*P*-value
< 5000	Referent	
5000–9999	−0.04 (0.02)	0.06
10 000–49 999	−0.06 (0.02)	0.002
50 000–99 999	−0.01 (0.03)	0.71
≥100 000	−0.06 (0.03)	0.04
CD4 count/μl		
> 500	Referent	
350–500	−0.02 (0.02)	0.18
200–349	−0.05 (0.02)	0.01
< 200	−0.04 (0.02)	0.07

[a]β-estimates (SE) from generalized estimating equations represent mean differences from the referent group on the outcome variable.

Discussion

The effect of HAART on salivary gland function was assessed longitudinally in HIV positive women on HAART. Objective measurements of the salivary flow rates showed a significant decrease in the stimulated flow rates among HIV positive women on maintenance therapy of HAART. Xerostomia and/or oral ulcerations has been reported as a specific side effect of PI therapy in 7% of HIV positive patients (Scully and Diz Dios, 2001). Earlier studies have shown that considerable variation exists in the normal range of salivary flow in humans (Dawes, 1987; Ship *et al*, 1991). In order to determine if PI were responsible for the observed decrease in the salivary flow rates we examined PI and Non-PI based effects on the salivary flow rates. Our analysis clearly showed that PI based HAART was significantly associated with a decrease in both unstimulated and stimulated salivary flow rates. Our findings are contradictory to those of Lin *et al* (2006) where no changes were observed in the salivary flow rates between HIV positive men on HAART and those not on HAART. The definition of a HAART user in their study was different from the definition used in our study. HAART users were not subdivided into non-users, stoppers, initiators or maintainers as in our study, and the effects of HAART on salivary flow rate were not specifically assessed between PI and Non-PI based HAART groups. Finally, the method of saliva collection differed from that used in our study and the study population was limited to men. Lin *et al* (2006) measured salivary flow by collecting whole unstimulated saliva and both unstimulated and stimulated saliva from individual major glands (Parotid; Submandibular and Sublingual together). These differences in the methodology of the study design and analysis of results may explain the lack of effect of HAART on salivary flow in their study.

Previously we had reported that HAART is a risk factor for low unstimulated and stimulated salivary flow rates (Navazesh *et al*, 2003). Salivary Leukocyte PI levels in the saliva have been shown to be increased

in patients on PI based HAART (Lin *et al*, 2004). This may also imply that PI levels are increased within the salivary glands. In this study, we have shown that PI based HAART alone is responsible for low unstimulated and stimulated salivary flow rates. At present, it is still unclear as to why PI based HAART therapy in maintainers might produce a significant decrease in the unstimulated and stimulated salivary flow rates. It is possible that the chemical structure of the PI somehow alter the structure and composition of saliva thereby decreasing the salivary flow (Navazesh *et al*, 2003). Another possibility by which PIs might affect the salivary flow rates is lipotrophic changes or deposition of adipose tissue within the gland itself.

Salivary gland enlargement, bilateral or unilateral and almost always affecting the parotid gland, has been infrequently reported as a manifestation of HIV infection (Patton *et al*, 2002). Before the advent of HAART, parotid gland enlargement had been reported to occur in 5–10% of the HIV-affected-population (Schiodt *et al*, 1989; Soberman *et al*, 1991; Mandel and Surattanont, 2002) and the glandular enlargement was traditionally correlated with decreased CD4 levels and advanced stages of HIV infection or AIDS (Vargas *et al*, 2003). In our study, women with an AIDS outcome were shown to have a 60% greater risk for having enlarged salivary glands (data not shown). With the introduction of HAART therapy in the mid-1990s a decline in the prevalence of oral manifestations of HIV infection and AIDS has been reported in the literature (Patton *et al*, 2000, 2002; Greenspan and Greenspan, 2002). Overall, there appears to be a reduced prevalence of oral candidiasis, Kaposi's sarcoma, oral hairy leukoplakia, and HIV-associated periodontal disease in adults. However, there has been an increase in HPV-associated oral lesions (papillomas, condylomas and focal epithelial hyperplasia) and HIV-related salivary gland disease in the developed world (Schmidt-Westhausen *et al*, 2000; Greenspan *et al*, 2001; Reichart, 2003, 2006). Patton *et al* (2000) reported a significant increase in the prevalence of HIV-associated salivary gland disease from l.8% to 5% in their study. Our findings show that HAART use, particularly PI based HAART, is a significant risk factor for developing enlarged salivary glands. These findings are contradictory to the findings from developing countries like Mexico, where no changes were recorded in the prevalence of salivary gland enlargement in 1000 HIV infected individuals (87.9% male; 12.1% female) (Ramirez-Amador *et al*, 2003). Nicolatou-Galitis *et al*, (2004) reported that there was no change in the prevalence of salivary gland disease in a group of 95 HIV infected Greek individuals (10 females, 85 males) on HAART therapy with a PI. This result may be due to a small sample size. As mentioned earlier Panayiotakopoulos *et al* (2003) and Basu *et al* (2006) have shown a decrease in the prevalence of DILS. Given the previous outcomes, it remains unclear whether salivary gland enlargement, especially of the parotid glands is on the rise in HIV infected individuals on HAART. Our findings show that HAART use in a female population, particularly PI based HAART, is a risk factor for developing salivary gland enlargement.

In early 1998, Carr *et al* provided a detailed description of a syndrome of peripheral lipodystrophy, dyslipidemia, and insulin resistance related to the use of PI. Subsequently, several reports were published on the association of PI based HAART therapy and lipodystrophy in HIV infected individuals and the condition is referred to as lipodystrophy syndrome in HIV infected individuals (LDHIV) (Kravcik, 2000). PI cause fat accumulation in various parts of the body such as the back of the neck (buffalo hump) and intra-abdominal region. As mentioned earlier, PIs have also been suggested to cause fatty infiltration of the parotid gland or parotid lipomatosis resulting in glandular swelling (Aguirre-Urizar *et al*, 2004). Parotid lipomatosis was first reported by Olive *et al* (1998) as a side effect of PI therapy. Ceballos-Salobrena *et al* (2000) reported parotid gland enlargement in HIV positive patients (4.5%) on HAART. Although this finding did not demonstrate a statistically significant association to HAART usage in their study, they suggested that HIV parenchymatous infiltration in the early stages as well as lipodystrophic changes secondary to PI based HAART usage in certain AIDS patients may be responsible for the observed enlargement.

The underlying pathogenesis for the lipodystrophy syndrome is unclear and the precise mechanisms by which PI induce these changes are unknown. Several mechanisms have been proposed to be involved in causing lipid accumulation and atrophy in the central and peripheral tissues respectively. Carr *et al* (1998) compared the 12 amino acid genetic sequence of the catalytic region of HIV-1 protease with all mammalian protein and genome sequences in gene libraries. A 60% homology was found with two proteins that regulate lipid metabolism: cytoplasmic retinoic acid binding protein type 1 (CRABP-1) and low-density lipoprotein receptor-related protein (LRP). CRABP-1 carries retinoic acid (Li and Norris, 1996) which, when isomerized to *cis*-9-retinoic acid, activates nuclear retinoid X receptor-α-peroxisome proliferator-activated receptor-γ (PPAR-γ) complex known to regulate adipocyte proliferation and differentiation (Tontonoz *et al*, 1995; Chambon, 1996). Furthermore Carr *et al* (1998) hypothesized that PI inhibit CRABP-1 and LRP and therefore produce lipodystrophic changes. However, three dimensional X-ray crystallography of CRABP-1 and HIV-1 protease showed significant differences, meaning PI will not be able to inhibit conversion of *trans* to *cis*-9-retinoic acid (Stevens *et al*, 1999). In addition, PI have not been shown to bind to the PPAR-γ complex. Studies in mice have failed to produce hyperlipidemia following inhibition of LRP by PI (Rohlmann *et al*, 1998).

On an average, about 5–10 intra-parotid lymph nodes are usually distributed throughout the parotid gland. These nodes become trapped within the parotid gland during embryologic development (Ioachim *et al*, 1988; Mandel and Hong, 1999). In the HAART era, parotid gland swelling has been attributed to the increase in the

CD8 lymphocyte count with CD8 lymphocytic infiltration into the intra-parotid lymph nodes in patients receiving HAART therapy. Intra-parotid lymph nodes undergo a significant hyperplastic response and lead to the visible parotid enlargement (Itescu *et al*, 1990; Mandel and Surattanont, 2002). This lymphoproliferation probably reflects a reaction to the presence of high concentration of HIV p24 antigen in the gland (Bruner *et al*, 1989; Vicandi *et al*, 1999; Uccini *et al*, 2000). Ceballos-Salobrena *et al* (2000) reported an overall decrease in the prevalence of oral lesions except xerostomia (15.5% *vs* 4%) in a group of HIV positive patients on HAART along with a strong relationship between the quantity of viral load and prevalence of oral lesions. Also, increased viral loads greater than 10 000 copies were significantly associated with an increased prevalence of xerostomia (29.7%). In their study, reduced CD4 cell counts were not shown to be associated with the increased prevalence of xerostomia. Our findings also showed that increased HIV RNA levels were significantly associated with reduced unstimulated (for viral load >5000) and stimulated (for viral load $>10\,000$) salivary flow rates (data not shown). It is possible that the lymphoproliferative response as a result of high levels of HIV p24 antigen may have been an important risk factor for developing reduced salivary flow rates and salivary gland enlargement in our study. Our findings also showed that reduced CD4 cell counts (<350) were significantly associated with a decrease in the unstimulated salivary flow rate. Our study did not include any imaging or histologic evaluation of the salivary glands therefore, the exact nature (functional or structural) of associated changes with HAART remains unknown.

An earlier report of a larger sample size of HIV infected women ($n = 1256$) revealed the prevalence of various clinical symptoms in HIV positive women responding to a questionnaire (Silverberg *et al*, 2004). The prevalence of xerostomia was found to be increased in patients who had discontinued HAART (18.7%) and those who had switched HAART regimens (18.4%). The lowest prevalence of xerostomia was reported in patients with stable HAART use (13.1%). Similar findings were observed in our study where continued HAART usage for at least 6 months decreased the risk for developing a complaint of too little saliva. Paradoxically, in our study, PI based HAART maintainers had a significant decrease in both unstimulated and stimulated salivary flow rates. The lack of a subjective report of dry mouth in the presence of objective evidence of decreased salivary flow was observed in HAART maintainers. This finding is not unique for our study population. In fact, several studies have clearly shown the wide variability in unstimulated and stimulated salivary flow rates in healthy adults that are adequate for normal oral function (Dawes, 1987; Ship *et al*, 1991). The explanation for such variability is unknown. HAART use was not found to be associated with tenderness or lack of secretion on palpation of the salivary glands.

Saliva plays a significant role in oral and systemic health and its absence affects the quality of life. Individuals who suffer from salivary gland dysfunction are at risk for development of dental caries, periodontal diseases and fungal infection (Navazesh *et al*, 2002). A variety of medical conditions and medications can contribute to the development of salivary gland dysfunction. In our study, we attempted to control for all potential known factors that could have contributed to a complaint of dry mouth and or reduced saliva flow rates. PI based HAART therapy emerged to be a significant and independent risk factor for salivary gland dysfunction in HIV positive women.

Acknowledgements

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.

Author contributions

M Navazesh is the lead author for this study and was the lead person in charge of supervising the co-authors in the design, planning, quality assurance, monitoring the progress of the study, review the collected data, literature review, data analysis and manuscript preparation and review. R Mulligan, J Greenspan, D Greenspan, J Phelan and M Alves helped with the design, planning, quality assurance, monitoring the progress of the study as well as review the collected data and manuscript. S Hazem helped with preliminary data analysis. R Karim and WJ Mack helped with data analysis and manuscript preparation. S Ram helped with the literature review and manuscript preparation.

References

Aguirre-Urizar JM, Echebarria-Goicouria MA, Eguia-del-Valle A (2004). Acquired immunodeficiency syndrome: manifestations in the oral cavity. *Med Oral Patol Oral Cir Bucal* **9**(Suppl.)**:** 153–157; 148–153.

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

Basu D, Williams FM, Ahn CW, Reveille JD (2006). Changing spectrum of the diffuse infiltrative lymphocytosis syndrome. *Arthritis Rheum* **55:** 466–472.

Bruner JM, Cleary KR, Smith FB, Batsakis JG (1989). Immunocytochemical identification of HIV (p24) antigen in parotid lymphoid lesions. *J Laryngol Otol* **103:** 1063–1066.

Carr A, Samaras K, Burton S, *et al* (1998). A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *Aids* **12:** F51–58.

Ceballos-Salobrena A, Gaitan-Cepeda LA, Ceballos-Garcia L, Lezama-Del Valle D (2000). Oral lesions in HIV/AIDS patients undergoing highly active antiretroviral treatment including protease inhibitors: a new face of oral AIDS? *AIDS Patient Care STDS* **14:** 627–635.

Chambon P (1996). A decade of molecular biology of retinoic acid receptors. *FASEB J* **10:** 940–954.

Dawes C (1987). Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J Dent Res* **66:** 648–653.

Greenspan J, Greenspan D (2002). The epidemiology of the oral lesions of HIV infection in the developed world. *Oral Dis* **8**(Suppl. 2)**:** 34–39.

Greenspan D, Canchola AJ, MacPhail LA, Cheikh B, Greenspan JS (2001). Effect of highly active antiretroviral therapy on frequency of oral warts. *Lancet* **357:** 1411–1412.

Ioachim HL, Ryan JR, Blaugrund SM (1988). Salivary gland lymph nodes. The site of lymphadenopathies and lymphomas associated with human immunodeficiency virus infection. *Arch Pathol Lab Med* **112:** 1224–1228.

Itescu S, Brancato LJ, Buxbaum J *et al* (1990). A diffuse infiltrative CD8 lymphocytosis syndrome in human immunodeficiency virus (HIV) infection: a host immune response associated with HLA-DR5. *Ann Intern Med* **112:** 3–10.

Kozinetz CA, Carter AB, Simon C *et al* (2000). Oral manifestations of pediatric vertical HIV infection. *AIDS Patient Care STDs* **14:** 89–94.

Kravcik S (2000). HIV lipodystrophy: a review. *HIV Clin Trials* **1:** 37–50.

Laskaris G, Hadjivassiliou M, Stratigos J (1992). Oral signs and symptoms in 160 Greek HIV-infected patients. *J Oral Pathol Med* **21:** 120–123.

Levine AM, Karim R, Mack W *et al* (2006). Neutropenia in human immunodeficiency virus infection: data from the women's interagency HIV study. *Arch Intern Med* **166:** 405–410.

Li E, Norris AW (1996). Structure/function of cytoplasmic vitamin A-binding proteins. *Annu Rev Nutr* **16:** 205–234.

Lin AL, Johnson DA, Stephan KT, Yeh CK (2003). Alteration in salivary function in early HIV infection. *J Dent Res* **82:** 719–724.

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.

Lin AL, Johnson DA, Sims CA, Stephan KT, Yeh CK (2006). Salivary gland function in HIV-infected patients treated with highly active antiretroviral therapy (HAART). *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **102:** 318–324.

Mandel L, Hong J (1999). HIV-associated parotid lymphoepithelial cysts. *J Am Dent Assoc* **130:** 528–532.

Mandel L, Surattanont F (2002). Regression of HIV parotid swellings after antiviral therapy: case reports with computed tomographic scan evidence. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **94:** 454–459.

Mulligan R, Phelan JA, Brunelle J *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, 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, Denny P, Sobel S (2002). Saliva: a fountain of opportunity. *J Calif Dent Assoc* **30:** 783–788.

Navazesh M, Mulligan R, Barron Y *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.

Nicolatou-Galitis O, Velegraki A, Paikos S *et al* (2004). Effect of PI-HAART on the prevalence of oral lesions in HIV-1 infected patients. *A Greek study*. *Oral Dis* **10:** 145–150.

Olive A, Salavert A, Manriquez M, Clotet B, Moragas A (1998). Parotid lipomatosis in HIV positive patients: a new clinical disorder associated with protease inhibitors. *Ann Rheum Dis* **57:** 749.

Panayiotakopoulos GD, Aroni K, Kyriaki D *et al* (2003). Paucity of Sjogren-like syndrome in a cohort of HIV-1-positive patients in the HAART era. Part II. *Rheumatology (Oxford)* **42:** 1164–1167.

Patton LL, McKaig R, Strauss R, Rogers D, Eron JJ Jr (2000). Changing prevalence of oral manifestations of human immuno-deficiency virus in the era of protease inhibitor therapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **89:** 299–304.

Patton LL, Phelan JA, Ramos-Gomez FJ, Nittayananta W, Shiboski CH, Mbuguye TL (2002). Prevalence and classification of HIV-associated oral lesions. *Oral Dis* **8**(Suppl. 2)**:** 98–109.

Ramirez-Amador V, Esquivel-Pedraza L, Sierra-Madero J, Anaya-Saavedra G, Gonzalez-Ramirez I, Ponce-de-Leon S (2003). The Changing Clinical Spectrum of Human Immunodeficiency Virus (HIV)-Related Oral Lesions in 1,000 Consecutive Patients: A 12-Year Study in a Referral Center in Mexico. *Medicine (Baltimore)* **82:** 39–50.

Ramos-Gomez FJ, Flaitz C, Catapano P, Murray P, Milnes AR, Dorenbaum A (1999). Classification, diagnostic criteria, and treatment recommendations for orofacial manifestations in HIV-infected pediatric patients. Collaborative Workgroup on Oral Manifestations of Pediatric HIV Infection. *J Clin Pediatr Dent* **23:** 85–96.

Reichart PA (2003). Oral manifestations in HIV infection: fungal and bacterial infections, Kaposi's sarcoma. *Med Microbiol Immunol* **192:** 165–169.

Reichart P (2006). US1 HIV – changing patterns in HAART era, patients' quality of life and occupational risks. *Oral Dis* **12**(Suppl. 1)**:** 3.

Rohlmann A, Gotthardt M, Hammer RE, Herz J (1998). Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest* **101:** 689–695.

Schiodt M (1997). Less common oral lesions associated with HIV infection: prevalence and classification. *Oral Dis* **3**(Suppl. 1)**:** S208–S213.

Schiodt M, Greenspan D, Daniels TE *et al* (1989). Parotid gland enlargement and xerostomia associated with labial sialadenitis in HIV-infected patients. *J Autoimmun* **2:** 415–425.

Schmidt-Westhausen AM, Priepke F, Bergmann FJ, Reichart PA (2000). Decline in the rate of oral opportunistic infections following introduction of highly active antiretroviral therapy. *J Oral Pathol Med* **29:** 336–341.

Scully C, Diz Dios P (2001). Orofacial effects of antiretroviral therapies. *Oral Dis* **7:** 205–210.

Ship JA, Fox PC, Baum BJ (1991). How much saliva is enough? 'Normal' function defined *J Am Dent Assoc* **122:** 63–69.

Silverberg MJ, Gore ME, French AL *et al* (2004). Prevalence of clinical symptoms associated with highly active antiretroviral therapy in the Women's Interagency HIV Study. *Clin Infect Dis* **39:** 717–724.

Soberman N, Leonidas JC, Berdon WE *et al* (1991). Parotid enlargement in children seropositive for human immunodeficiency virus: imaging findings. *AJR Am J Roentgenol* **157:** 553–556.

Stevens GJ, Grecko R (1999). Preclinical investigations into the mechanism by which HIV protease inhibitors may induce metabolic disorders [abstract]. *39th Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, CA.

Tontonoz P, Hu E, Spiegelman BM (1995). Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. *Curr Opin Genet Dev* **5:** 571–576.

Uccini S, D'Offizi G, Angelici A *et al* (2000). Cystic lymphoepithelial lesions of the parotid gland in HIV-1 infection. *AIDS Patient Care STDs* **14:** 143–147.

US Department of Health and Human Services (2002). *Henry J. Kaiser Family Foundation. Guidelines for the use of Antiretroviral Agents in HIVinfected Adults and Adolescents*. HIV/AIDS Treatment Information Service: Rockville, MD: Available at: http://aidsinfo.nih.gov/guidelines [accessed on 20 February 2008].

Vargas PA, Mauad T, Bohm GM, Saldiva PH, Almeida OP (2003). Parotid gland involvement in advanced AIDS. *Oral Dis* **9:** 55–61.

Vicandi B, Jimenez-Heffernan JA, Lopez-Ferrer P *et al* (1999). HIV-1 (p24)-positive multinucleated giant cells in HIV-associated lymphoepithelial lesion of the parotid gland. A report of two cases. *Acta Cytol* **43:** 247–251.

Yeh CK, Fox PC, Ship JA *et al* (1988). Oral defense mechanisms are impaired early in HIV-1 infected patients. *J Acquir Immune Defic Syndr* **1:** 361–366.

Yeni PG, Hammer SM, Carpenter CC *et al* (2002). Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *JAMA* **288:** 222–235.