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ORIGINAL ARTICLE

Salivary secretion, mucin concentrations and candida carriage in HIV-infected patients

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OBJECTIVES: To test whether the submandibular/sublingual (SMSL) salivary secretion, mucin concentration and candida carriage status were altered in human immunodeficiency virus-positive (HIV+) patients.

SUBJECTS AND METHODS: SMSL saliva collected from 48 HIV-infected and 31 HIV-negative men were analyzed for flow rates, total protein and mucin concentrations. Salivary cultures were performed for *Candida* assessment.

RESULTS: The salivary flow rate and protein secretion of the HIV+ patients was 37% and 32% less than that of the controls ($P < 0.0001$, $P = 0.0087$). The mucin concentrations (MG1 and MG2) were higher in the HIV+ subjects compared with controls ($P = 0.0186$, $P = 0.0014$); however, the mucin secretions were not different. The frequency of *Candida*-positive cultures was higher in the HIV+ subjects than in the controls (61.4% *vs* 24.1%, $P = 0.0018$). In the HIV-infected group, the unstimulated SMSL flow rates were lower in *Candida*-positive than in *Candida*-negative patients ($P = 0.0158$).

CONCLUSION: The salivary secretion of the SMSL glands was reduced in HIV infection. Although the mucin concentration increased in HIV+ subjects, mucin secretion was not altered. Highly active antiviral therapy had no effect on salivary function. We found an association between the level of candida carriage and salivary flow rate in HIV-infected patients.

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Keywords: mucin; saliva; HIV; *Candida*

Introduction

The risk of transmission of the human immunodeficiency virus (HIV) via saliva is low. This evidence has been confirmed by *in vitro* studies showing that saliva contains factors that inhibit HIV infectivity (Fox *et al*, 1988; Yeh *et al*, 1992; Bergey *et al*, 1993). Anti-HIV activities are found to be more potent and consistent in submandibular/sublingual (SMSL) and whole saliva than in parotid saliva (Nagashunmugam *et al*, 1997). Filtration of SMSL and whole saliva has been shown to reduce the antiviral activity, suggesting that large-molecular weight compounds present in saliva that are retained by the filter may inhibit HIV infectivity (Yeh *et al*, 1992; Bergey *et al*, 1993).

Human salivary mucins have a multifunctional role in the oral cavity. They lubricate oral mucosal surfaces and provide a protective barrier and aid in mastication, speech and swallowing (Mandel, 1987; Tabak, 1990). Mucins are glycoproteins with low solubility, high viscosity and adhesiveness. Salivary mucins are components of the innate host defense system of the oral cavity. As salivary mucins play major roles in the protection of the oral tissues, lower levels of these mucins may cause long-term negative effects on oral health (Baughan *et al*, 2000). There are two types of mucin in saliva: oligomeric mucin glycoprotein (MG1) and monomeric mucin glycoprotein (MG2) (Zalewska *et al*, 2000). Rayment *et al* (2000) have shown that the concentration of MG1 is greater than that of MG2 and that salivary mucins constitute approximately 16% of the total protein content in whole saliva. MG1 is a mixture of mucin gene products consisting predominantly of MUC5B and, to a lesser extent, MUC4 (Nielsen *et al*, 1997; Troxler *et al*, 1997; Liu *et al*, 1998; Thornton *et al*, 1999). MG2 is the product of the MUC7 gene. MG1 is present in the mucous acini of submandibular, sublingual, labial and palatine salivary glands (Cohen *et al*, 1990; Nielsen *et al*, 1997) whereas MG2 is present in the mucous acini of submandibular and labial salivary glands (Cohen *et al*, 1991) and in serous acini of submandibular, sublingual, labial and palatine salivary glands (Nielsen *et al*, 1996). The major function of MG1 is to coat and protect the oral tissues. A reduction in the MG1 concentration may make the coating of MG1 on the mucosal surfaces difficult which leads to a reduction

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in the protection of the oral tissues and may increase host susceptibility to mucosal damage (Almstahl *et al*, 2001). MG2 interacts with the oral flora by promoting their agglutination and clearing of the oral cavity (Levine *et al*, 1978; Tabak, 1990; Scannapieco, 1994). Elevated *Streptococcus mutans* titers are significantly associated with diminished concentrations of MG2 in unstimulated whole saliva (Baughan *et al*, 2000).

Studies have indicated that HIV is entrapped mainly by salivary mucins. Habte *et al* (2006) reported the anti-HIV-1 activities of human crude saliva and purified salivary MUC5B and MUC7 mucins in an *in vitro* inhibition assay. They showed that both crude saliva and purified MUC5B and MUC7 mucins inhibit HIV-1 activity by 100% in the range of 900–0.09 μg mucin concentration. This study has shown that mucus and mucins have protective properties against the HIV virus.

Oral candidiasis is the most common mucosal lesion reported in HIV-infected patients (Greenspan *et al*, 2000; Reichart, 2003). It is suggested that altered salivary gland function predisposes HIV-infected patients to oral candidiasis (Pollock *et al*, 1992; Lin *et al*, 2001). The severity and prevalence of oral candidiasis increase with advancing immune suppression (Klein *et al*, 1984). There appears to be an association between oral candidiasis and the number of $CD4^+$ cells (Ohmit *et al*, 2003; Yang *et al*, 2006). The mechanisms that inhibit the ability of *Candida* to adhere to mucous membrane or to replicate and/or to form hyphae may prevent oral candidiasis (Enwonwu and Meeks, 1996). Other mechanisms that may aid in the control of oral *Candida* infection are the cleansing mechanism of saliva as well as specific protein components. Mucins appear to play an important role in the aforementioned mechanisms. Situ *et al* (2003) have shown that human salivary MUC7 mucin peptides exhibit antifungal activity against *Candida albicans*. Ogasawara *et al* (2007) have demonstrated that mucin inhibits hyphal formation in *C. albicans*. These studies suggest that mucin may act as an inhibitor to candida infection.

We and others have reported salivary hypofunction with alterations in compositions and anti-candida activities in HIV+ populations (Yeh *et al*, 1988; Atkinson *et al*, 1989; Mandel *et al*, 1992; Lin *et al*, 2003, 2006; Navazesh *et al*, 2003). To our knowledge, there is no publication regarding the concentration of salivary mucins in HIV-infected patients. Therefore, the purposes of our study were to (1) determine the mucin concentration of the SMSL glands in HIV-positive (HIV+) patients, (2) investigate the effect of HIV infection and highly active antiviral therapy (HAART) on salivary mucin concentration and candida carriage status and (3) determine any correlation between the mucin concentration and the candida carriage status.

Subjects and methods

Subjects

Forty-eight HIV+ men who are participants in the US Air Force HIV Natural History Study and the NIH-funded longitudinal study of 'Saliva and Anticandidal Defense Mechanisms in HIV/AIDS' were recruited. Forty-seven patients had a $CD4^+$ cell count >200 cells μl^{-1}. The HIV+ subjects were divided into two subgroups based on whether or not they were receiving HAART. A patient is considered to be HAART-positive (HAART+) if he is taking a combination of a protease inhibitor plus two other nucleoside or non-nucleoside reverse transcriptase inhibitors. $CD4^+$ cell counts of the patients on or near the date of saliva sample collection were obtained from their medical records. Thirty-one HIV-negative (HIV−) men of a comparable age range, who were not taking medications or smoking, were included as the control group. This study was approved by the University of Texas Health Science Center at San Antonio and the Wilford Hall Air Force Medical Center Institutional Review Boards. Informed consent was obtained from all subjects as required by Air Force Regulation 169-9 and University of Texas policies.

Saliva collection and analytical methods

Unstimulated and stimulated SMSL salivary samples were collected from subjects according to a previous published protocol (Yeh *et al*, 1988). For collection of SMSL saliva, the orifices of Wharton's and Bartholin's ducts were isolated with cotton rolls and saliva was collected with gentle suction as it emerged from the orifices. Gland stimulation was achieved by swabbing the dorsolateral tongue with 2% citric acid solution every 30 s. The SMSL salivary flow rate was calculated as the weight of saliva divided by the collection time and then subdivided by 2 to give a flow rate of ml min^{-1} per gland. Each salivary sample was divided into 100 μl aliquots and stored at −70°C until analyzed. Stimulated SMSL saliva was analyzed for total protein and mucins. Total protein in saliva was determined by absorption at 215 nm with bovine serum albumin as a standard (Arneberg, 1971). Mucins (MG1 and MG2) were quantified using a polyacrylamide gel separation technique (Denny *et al*, 1991) followed by staining with periodic acid Schiff reagent. Gels were loaded with equal amount of salivary proteins. After electrophoresis, gels were scanned with a densitometric scanner and NIH Image (version 1.47) was used for the analysis. Within each gel, three different volumes of a SMSL saliva pool were included to provide a standard curve of color units against which the concentration of mucins was determined as unit ml^{-1}. The original saliva sample volume was used to convert mucin concentration to unit ml^{-1}. Mucin secretion (unit min^{-1}) was obtained from the value of mucin concentration multiplied by flow rate.

Candida assessment

Serial dilutions of unstimulated whole saliva samples (20 μl) were spread onto Sabouraud's agar plates containing chloramphenicol (100 $\mu g\ ml^{-1}$) and incubated at 37°C. After 30 h of incubation, the candida colony forming units (CFU) on each agar plate were counted, averaged and expressed as CFU ml^{-1}.

Statistical analysis

The nonparametric Mann–Whitney *U*-test was used to analyze the differences in $CD4^+$ cell and candida counts between groups. The chi-squared test was used to test the differences in candida carriage rates between HIV+ and the control groups as well as between the HAART subgroups. The unpaired *t*-test was used to determine the differences on continuous variables between groups. The data on mucins were square-root-transformed prior to analysis. The significance level of difference was set at $P < 0.05$.

Results

Table 1 illustrates the mean of age and median (with the 25th to 75th percentiles in parentheses) of $CD4^+$ cell counts in HIV+ and control groups. There were no differences in mean age between the HIV+ patients and the controls. However, the patients in the HAART+ subgroup were slightly older than those in the HAART− subgroup and the control group ($P = 0.0349$ and 0.0239, respectively). There were no differences in the median number of $CD4^+$ cells between the HAART+ and HAART− subgroups.

Table 2 lists the stimulated SMSL salivary flow rates, total protein concentrations and secretions in HIV+ patients and controls. The mean salivary flow rate of the HIV+ patients was 37% less than that of the controls ($P < 0.0001$). The salivary flow rates were not different between the two HAART subgroups. No difference in protein concentration was observed between the HIV+ patients and controls or between the two HAART subgroups. In HIV+ patients, protein secretion was 32% less than that of the controls ($P = 0.0087$). Between the two HAART subgroups, the protein secretions were not different.

Table 1 Age and $CD4^+$ cell counts in HIV+ and control groups

Group	*Age (years)*	*$CD4^+$ (cell μl^{-1})*
Control ($n = 31$)	32.2 ± 1.3	NA
HIV+ ($n = 48$)	34.3 ± 1.1	546 ± 35 (358–726)
HAART+ ($n = 20$)	36.9 ± 1.6*	505 ± 51 (303–585)
HAART− ($n = 28$)	32.4 ± 1.4*	575 ± 47 (391–745)

Data for age are mean ± standard error, while those for $CD4^+$ cell counts are median ± standard error, with 25th to 75th percentiles in parentheses. HIV, human immunodeficiency virus; HAART, highly active antiviral therapy; NA, non-available.
*$P = 0.0349$.

Table 3 presents the salivary mucin concentrations and mucin secretions in HIV+ patients and controls. Statistical analysis showed significantly higher MG1 (44%, $P = 0.0186$) and MG2 (55%, $P = 0.0014$) concentrations in HIV+ patients compared with controls. The mucin secretions for MG1 and MG2 were not different between the HIV+ and control groups. Also, there were no differences in mucin concentrations and secretions observed between the two HAART subgroups.

Table 4 compares the candida carriage status and candida count in whole saliva of HIV+ and control groups. *Candida* cultures were not available in two controls and in four HIV+ patients. The frequency of *Candida*-positive (*Cand*+) cultures for HIV+ patients was higher compared with controls (61.4% *vs* 24.1%, $P = 0.0018$). The carriage rate was also higher in the two HAART subgroups in comparison with the control group ($P = 0.0038$ for HAART+ and $P = 0.0112$ for HAART−). However, the carriage rate between the two HAART subgroups was not significantly different. Among those who were carriers, the median candida count was higher in the HIV+ patients than in the controls (880 *vs* 400 CFU ml^{-1}), but this was not statistically significant.

Table 5 shows the salivary flow rates, mucin concentrations and secretions and $CD4^+$ cell counts in relation to candida carriage status. In the HIV-infected group, negative associations between candida carriage status and flow rate and $CD4^+$ cell count were noted. The unstimulated SMSL flow rate in the *Cand*+ patients was lower than that in the *Cand*− patients ($P = 0.0158$). The $CD4^+$ cell count was also lower in *Cand*+ than in *Cand*− patients ($P = 0.0326$). No differences in the mucin concentrations and secretions were found between the *Cand*+ and *Cand*− subjects both in the HIV+ and control groups.

Discussion

We chose to conduct the study using SMSL saliva because it comprises most of the saliva in the oral cavity (Schneyer, 1956) and changes in SMSL saliva may have significant oral implications (Mandel, 1987). Atkinson *et al* (1989) performed a longitudinal study and demonstrated that SMSL function is affected earlier from HIV infection than parotid function. Also, SMSL saliva has a higher anti-HIV activity than does parotid or whole saliva (Nagashunmugam *et al*, 1997). Reduced salivary flow rates have been reported in most HIV/AIDS studies

Table 2 Stimulated submandibular/sublingual flow rates, total protein concentrations and secretions in HIV+ and control groups

Group	*Flow rate (ml min^{-1} per gland)*	*Protein concentration (mg ml^{-1})*	*Protein secretion (mg min^{-1})*
Control	0.49 ± 0.03 ($n = 31$)*	2.24 ± 0.15 ($n = 31$)	1.14 ± 0.12 ($n = 31$)**
HIV+	0.31 ± 0.02 ($n = 48$)*	2.31 ± 0.11 ($n = 46$)	0.77 ± 0.08 ($n = 46$)**
HAART+	0.30 ± 0.03 ($n = 20$)	2.30 ± 0.18 ($n = 19$)	0.70 ± 0.08 ($n = 19$)
HAART−	0.32 ± 0.03 ($n = 28$)	2.31 ± 0.15 ($n = 27$)	0.83 ± 0.12 ($n = 27$)

Data expressed as mean ± standard error with the number of subjects tested in parentheses. HIV, human immunodeficiency virus; HAART, highly active antiviral therapy.
*$P < 0.0001$, **$P = 0.0087$.

Table 3 Salivary mucin concentrations and secretions in HIV+ and control groups

	Mucin concentration (unit ml^{-1})		*Mucin secretion (unit min^{-1})*	
Group	*MG1*	*MG2*	*MG1*	*MG2*
Control (n = 31)	0.39 ± 0.04*	0.62 ± 0.05**	0.17 ± 0.02	0.30 ± 0.03
HIV+ (n = 48)	0.56 ± 0.06*	0.96 ± 0.08**	0.18 ± 0.02	0.29 ± 0.03
HAART+ (n = 20)	0.62 ± 0.11	1.02 ± 0.14	0.17 ± 0.02	0.29 ± 0.04
HAART− (n = 28)	0.51 ± 0.06	0.91 ± 0.09	0.16 ± 0.02	0.29 ± 0.04

Data expressed as mean ± standard error. HIV, human immunodeficiency virus; HAART, highly active antiviral therapy.
*P = 0.0186, **P = 0.0014.

Table 4 Candida carriage status and candida count in whole saliva of HIV+ and control groups

			Candida count (CFU ml^{-1})		
Group	*Cand−, n* (%)	*Cand+, n* (%)	*≤ 1000, n* (%)	*> 1000, n* (%)	*Median*
Control (n = 29)	22 (75.9)	7 (24.1)*	5 (71.4)	2 (28.6)	400
HIV+ (n = 44)	17 (38.6)	27 (61.4)*	14 (51.8)	13 (48.1)	880
HAART+ (n = 18)	6 (33.3)	12 (66.7)	6 (50.0)	6 (50.0)	765
HAART− (n = 26)	11 (42.3)	15 (57.7)	8 (53.3)	7 (46.7)	880

Candida cultures were not available in two controls and four HIV+ patients. HIV, human immunodeficiency virus; HAART, highly active antiviral therapy.
*P = 0.0018.

Table 5 The submandibular/sublingual flow rates, mucin concentrations and secretions and CD4+ cell count in HIV+ and control groups in relation to candida carriage status

	Control group		*HIV+ group*	
	Cand+ (n = *7*)	*Cand−* (n = *22*)	*Cand+* (n = *27*)	*Cand−* (n = *17*)
Unstimulated flow rate (ml min^{-1} per gland)	0.10 ± 0.03	0.14 ± 0.02	0.08 ± 0.01*	0.15 ± 0.02*
Stimulated flow rate (ml min^{-1} per gland)	0.48 ± 0.05	0.49 ± 0.03	0.29 ± 0.03	0.33 ± 0.04
MG1 concentration (unit ml^{-1})	0.47 ± 0.11	0.35 ± 0.04	0.52 ± 0.09	0.61 ± 0.07
MG2 concentration (unit ml^{-1})	0.63 ± 0.09	0.59 ± 0.06	0.92 ± 0.10	0.98 ± 0.13
MG1 secretion (unit min^{-1})	0.23 ± 0.05	0.16 ± 0.02	0.14 ± 0.02	0.21 ± 0.03
MG2 secretion (unit min^{-1})	0.31 ± 0.05	0.28 ± 0.03	0.24 ± 0.03	0.34 ± 0.06
CD4$^+$ cell count (cell μl^{-1})	NA	NA	470 ± 40**	621 ± 59**

Data expressed as mean ± standard error. NA, non-available.
*P = 0.0158, **P = 0.0326.

(Yeh *et al*, 1988; Atkinson *et al*, 1990; Mandel *et al*, 1992; Lin *et al*, 2001, 2003; Navazesh *et al*, 2003). The effect of HIV infection on salivary gland function in HIV+ patients is still unclear. Several factors including xerostomic medications, immunosuppression, cytomegalovirus co-infection and HAART have been implicated (Greenberg *et al*, 1997; Navazesh *et al*, 2000). However, Lin *et al* (2003) failed to relate salivary gland hypofunction in HIV-infected patients to the use of xerostomic medications. Recently, an increased occurrence of salivary gland enlargement and dry mouth has been associated with HAART as part of a phenomenon called immune restoration or reconstitution disease (Hirsch *et al*, 2004; Navazesh *et al*, 2005).

In the present study, the stimulated SMSL flow rate in HIV+ patients was reduced by 37% compared with controls. The protein secretion in HIV+ subjects was also reduced by 32% compared with controls. These findings confirm that the secretory function of SMSL glands is reduced in HIV infection and that the salivary composition is altered. We observed a negative association between the candida carriage status and flow rate in HIV-infected patients. This finding supports the theory that altered salivary function predisposes patients to oral candidiasis (Yeh *et al*, 1988; Jainkittivong *et al*, 1998; Lin *et al*, 2001). The etiology of oral candidiasis is more likely to be multifactorial (Ellepola and Samaranayake, 2001). Thus, in these HIV cohorts, it is not only hyposalivary function but also immunosuppression that predisposes patients to candida infection. This is supported by our findings in that the number of CD4$^+$ cells in HIV-infected patients was lower in *Cand+* patients than that in *Cand−* patients.

With regard to mucin concentration, the levels of MG1 and MG2 were higher in the HIV+ subjects than

in the controls. However, there were no significant differences in the secretory rates of MG1 and MG2 between the HIV+ patient and control groups. Therefore, the higher concentration of mucins in HIV+ patients was attributed to the reduction in flow rate. Both in the HIV+ and control groups, the concentrations of MG2 were greater than those of MG1; this is contradictory to the finding of Rayment *et al* (2000). This difference may be due to a different source of mucins and the quantification techniques used. Rayment *et al* (2000) reported no correlation between mucin concentration and flow rate whereas our study did. Salivary flow rate, protein concentration, protein secretion and mucin concentration were not different between the two HAART subgroups. This may imply that HAART exerts no effect on salivary gland function.

The candida carriage rate of the HIV+ patients in our study (61.4%) was comparable to that of other studies reporting carriage rates of 54.8–81.3% (Teanpaisan and Nittayananta, 1998; Tsang and Samaranayake, 2000; Campisi *et al*, 2002; Patel *et al*, 2006). The $CD4^+$ cell count is commonly used as a marker for immunosuppression and disease progression in HIV-infected patients. In our sample, only one patient had a $CD4^+$ cell count less than 200. The median value for the $CD4^+$ cell count indicates that the patients in this study were in the early stage of HIV infection. This may explain why the candida carriage status in the present study was not as high as those reported in other studies which were conducted on patients who were in a later stage of HIV infection. Patel *et al* (2006) showed the highest carriage rate and explained that their patients had lower $CD4^+$ cell counts and did not have access to HAART. Our findings showed no significant differences in candida carriage rate and candida count between the two HAART subgroups. This observation, as in our previous report (Lin *et al*, 2006), is in contrast to several studies reporting that HAART reduces candidiasis and candida carriage in HIV-infected patients (Navazesh *et al*, 2005; Yang *et al*, 2006). It is suggested that HAART suppresses HIV replication and increases the $CD4^+$ cell count. We found no difference in the $CD4^+$ cell count between the two HAART subgroups. This may be explained by the fact that our patients were in the early stage of HIV infection and thus the differences may be too small to be significant.

We did not find an association between the mucin concentration and candida carriage status as expected. Again, this may be because our HIV+ patients were in the early stage of infection and less than 50% of patients carried a high candida count. Thus, the association may be too weak to be detected. Studies of patients in later stages of HIV infection would probably give a better view of this association.

Conclusion

Based upon the results of this study, the salivary flow rate and protein secretion of SMSL glands were reduced in HIV infection. Although the mucin concentrations were increased in HIV infection, the mucin secretions were not altered. No effect of HAART was noted on salivary function. We confirm the negative association between the level of candida carriage and the $CD4^+$ cell count and salivary flow rate in HIV-infected patients.

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Author contributions

Jainkittivong A. analyzed the data and wrote the manuscript. Lin A. L. provided the verified dataset and assisted with data analysis. Johnson D. A. advised the authors on data analysis, interpretation and manuscript writing. Langlais R. P. critically reviewed and edited the manuscript. Yeh C-K. directed the study, finalized the manuscript and served as the corresponding author.

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