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Changes in oral cytokeratin expression in HIV-infected subjects with long-term use of HAART

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Abstract

Objectives—The objectives of this study were to determine 1) the expression of oral cytokeratins (CKs) among HIV-infected subjects compared to non-HIV controls, 2) the oral CKs expression in the subjects with HAART compared to those without HAART, and 3) factors associated with the expression of oral CKs.

Materials and methods—Oral tissues from buccal mucosa were obtained by punched biopsy in HIV-infected subjects with and without HAART, and non-HIV individuals. The samples were processed for immunohistochemical studies of CK1, CK13, CK14, CK16 and involucrin. The staining intensity was scored and recorded. Logistic regression analysis and multi-way ANOVA test were performed.

Results—The expression of CK13, CK14, and CK16 was found to be significantly different between HIV-infected subjects and non-HIV individuals ($p < .05$). The expression of those CKs was also significantly different between those who were and were not on HAART ($p < .05$). No significant difference between the groups was observed regarding CK1 and involucrin.

Conclusions—Oral epithelial cell differentiation as marked by the CKs expression is affected by HIV infection and use of HAART. CKs may be the useful biomarkers to identify HIV-infected subjects who are at risk of malignant transformation of the oral mucosa due to HIV infection and HAART.

Keywords

cytokeratin; HAART; HIV; oral health; oral lesion; risk factor

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Author contributions

Prof. Dr. Wipawee Nittayananta developed the proposal, applied for the research grant, directed the project, scored immunohistochemical staining, interpreted the results, prepared and revised a manuscript; Prof. Dr. Winyou Mitarnun advised and scored immunohistochemical staining, and prepared illustrations; Dr. Sineepat Talungchit performed clinical examination, punch biopsy and collected oral tissue samples; Assoc. Prof. Dr. Hutchia Sriplung contributed to data analysis.

Introduction

Highly active antiretroviral therapy (HAART) has become a standard treatment of the human immunodeficiency virus (HIV) infection. At present, HAART includes more than 30 different drugs of 6 separate classes: nucleoside reverse transcriptase inhibitors (NRTIs), non nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, entry inhibitors, and HIV integrase inhibitors (Hammer et al, 2008). Previous studies reported that HAART significantly decreased the prevalence of AIDS defining malignancy including Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL) (Simard *et al*, 2010; Seaberg *et al*, 2010). However, the incidence rates of non-AIDS defining cancers of different organs including oropharynx have progressively increased among those subjects with HAART (Gillison, 2009).

Cytokeratins (CKs) are intermediate filament cytoskeletal proteins divided into two subfamilies based on amino acid sequence and charge: Type I (acidic) and Type II (basic or neutral) (Presland and Dale 2000). Both types are coexpressed during the differentiation of epithelial tissues and arranged in heterotypic pairs to form chains of laterally aligned coiled-coil structure (Moll *et al*, 2008).

The assessment of the distribution of different CK subtypes can facilitate cell typing and identification (Sakamoto et al, 2011). In normal stratified squamous epithelia like buccal mucosa, the entire suprabasal compartment is comprised of CK4 and CK13, whereas the basal compartment is positive for CK5 and CK14 (Moll *et al*, 2008). CK subtyping is useful for cancer diagnosis, as cancer cells often exhibit abnormal CK expression profiles (Moll *et al*, 2008). Previous studies demonstrated that some specific CK subtypes are either down-regulated or up-regulated in oral epithelial dysplasia and oral squamous cell carcinoma (OSCC) (Fillies *et al*, 2006; Gires *et al*, 2006; Matthias *et al*, 2008; Toyoshima *et al*, 2008; Yanagawa *et al*, 2007; Zhong *et al*, 2007; Fillies *et al*, 2007). Thus, CKs may be used as biomarkers for early diagnosis and prognostic monitoring of OSCC (Wei *et al*, 2009).

HIV infection can promote the development of malignancy as a consequence of the immune depletion caused by the virus (Clifford *et al*, 2005). In addition, long-term use of HAART has the potential oncogenicity (Bedimo *et al*, 2008). Thus, HIV-infected subjects who are on long-term use of HAART are susceptible to develop malignancies including OSCC (Gillison 2009). Since the prevalence of OSCC is increased among HIV-infected subjects in HAART era (Silverberg *et al*, 2009), we hypothesized that the expression of oral CKs was altered among those who were on long-term use of HAART. The objectives of this study were to assess 1) the expression of oral CKs among HIV-infected subjects compared to non-HIV controls, 2) the oral CKs expression in HIV-infected subjects with HAART compared to those without HAART, and 3) factors associated with the expression of oral CKs.

Materials and methods

Subjects

A cross-sectional study was performed in HIV-infected subjects who came to receive HAART at the Internal Medicine Clinic at Songklanagarind Hospital and Hat Yai Regional

Hospital in southern Thailand. The inclusion criteria of subjects enrolled were i) seropositive for antibody to HIV when tested with a particle agglutination test for antibodies to HIV (SERODIA®-HIV, Fujirebio Inc., Shinjuku-ku, Tokyo, Japan) and enzyme-linked immunosorbent assay (ELISA) (Enzygnost® Anti-HIV ½ Plus, Behring, Behringwerke AG, Marburg, Germany), ii) currently taking HAART, and iii) willing to participate in the study. The exclusion criteria were i) severely ill HIV-infected subjects who could not cooperate with the procedures of tissue collection by punch biopsy, and 2) HIV-infected subjects who were at risk of prolong bleeding. HIV-infected individuals who came to those hospitals but had not yet started HAART, and non-HIV infected volunteer were asked to participate as controls.

Ethics

The study procedures were undertaken with the understanding and written consent of each subject and according to ethical principles, including the World Medical Association Declaration of Helsinki (version, 2002 www.wma.net/e/policy/b3.htm). The study protocol was also approved by the research committee at the Prince of Songkla University, and at the Ministry of Public Health, Thailand. All information about the patients and their identity were anonymous. Subjects were given both verbal and written information about the nature of the study. They were allowed to leave the study at any time during the procedures.

Clinical examination

History taking and oral examination were performed in HIV-subjects with and without ART and non-HIV individuals. Clinical diagnosis of HIV-related oral lesions was made according to the classified criteria (EC-Clearinghouse 1993; Shiboski et al, 2009). The following data were recorded; HIV status, duration of HIV infection (calculated from the time since HIV seropositivity was first diagnosed), use of HAART, duration of HAART, CD4⁺ cell count, HIV viral load, smoking habit and alcohol consumption.

Oral tissue collection

Tissue punch biopsy of 4 mm in diameter was performed under local anesthesia on clinically normal buccal mucosa of all HIV-infected subjects and non-HIV controls. The tissue samples were fixed in 10% formalin. Then, they were embedded in paraffin and undergone hematoxylin and eosin staining along with immunohistochemical staining of CK1, CK13, CK14, CK16, and involucrin using commercially available monoclonal antibodies, listed in Table 1.

Immunohistochemical staining of cytokeratins

Three to five micrometer sections of formalin fixed and paraffin embedded tissues were mounted on 3-aminopropyl tri ethoxysilane (APES) coated slides. Sections were de-waxed with xylene and rehydrated through a graded series of ethanol and finally in distilled water. Antigen was retrieved with pressure cooker in Tris EDTA buffer pH 9.0 for 30 sec. Blocking was performed with 3% hydrogen peroxide in absolute methanol for 35 min to quench the endogenous peroxidase activity of the tissues. The sections were then incubated with non-immune serum for 30 min in moisture chamber to block the non-specific binding

sites. Next, the sections were probed for the presence of different CKs using specific antibodies at appropriate dilutions (Table 1). The sections were stained by the modified labeled avidin-biotin technique with DAKO LSAB™ kit (DAKO Corporation, Carpinteria, CA, USA), and with chromogen 3,3' diaminobenzidine, then counterstained with Harris hematoxylin and mounted. Positive and negative controls were included in all batches. Normal buccal mucosa was used as positive controls for CK13, CK14, and involucrin, normal skin tissues for CK1, and SCC of lung for CK16.

Analysis of staining

The staining intensity of the epithelium (basal and suprabasal) was assessed and subjectively scored by two investigators (WM and WN) with complete agreement as follows: –, negative; +, weak; ++, moderate; +++, strong, and +++, very strong. Percents of staining cells were calculated as follows:

$$\frac{\text{number of cells with positive staining in target area}}{\text{total number of target cells}} \times 100$$

Statistical analysis

Descriptive statistics were used to analyze breakdown of subjects by status of HIV test and duration of HAART received. Chi-square test was employed to explore possible association between HIV/HAART status and staining of CKs. As the intensity of staining may be influenced by duration of HIV infection, duration of HAART, CD4⁺ cell count, HIV viral load, smoking and drinking behaviors, logistic regression was used to analyze the relationship between the expression of CKs and those factors. The difference of mean percent positive cells to CKs by various factors was tested by multi-way ANOVA method to adjust for other influencing factors. Statistical significance was set at 0.05.

Results

Demographic data and medical status

Tissue samples were obtained from 99 HIV-infected subjects receiving HAART (age range 23–57 yr, mean age 39 yr), 58 receiving no HAART (age range 20–59 yr, mean age 34 yr), and 50 non-HIV individuals (age range 19–59 yr, mean age 36 yr). Most HIV-infected subjects who were on HAART received no PI based regimen (n=84, 85%). Different combinations of HAART used among HIV-infected subjects were 2NRTIs+1NNRTI (n=82, 83%), 2NRTIs+2PIs (n=7, 7%), 2NRTIs+1PI (n=3, 3%), and others (n=7, 7%). Those who had been taking ART < 3 years were classified as a group with short-term use of HAART, and those who had been taking HAART for ≥ 3 years were classified as a group with long-term use of HAART, respectively. Demographic data and medical status of the subjects and controls are shown in Table 2.

Expression of cytokeratins in HIV-infected subjects compared with non-HIV controls

Changes in the expression of CKs were observed in HIV-infected subjects compared to non-HIV individuals (Table 3). CK13 was strongly expressed on the suprabasal layer with no

significant difference between the two groups. However, the expression of CK13 in the basal layer was significantly different between the groups ($p < 0.001$). More HIV-infected subjects had weak staining of CK13 in the basal layer than non-HIV individuals (22% vs 4%). All cases showed positive staining of CK14 in the basal layer. Figure 1 shows the different intensity of CK14 staining. The expression of CK14 and CK16 was found to be significantly different between HIV-infected subjects and non-HIV individuals ($p < 0.001$). CK16 was more expressed in HIV- infected subjects. However, it should be noted that it was a weak staining. No significant changes were observed in the expression of CK1 and involucrin.

Expression of cytokeratins in HIV-infected subjects with and without HAART

Changes in the expression of CKs were observed among HIV-infected subjects who received HAART and those who did not (Table 3). Although strong staining of CK13 was observed in the suprabasal layer of all subjects, the expression in the basal layer was found to be significantly different due to HAART status of the subjects ($p < 0.05$). More subjects who were on HAART showed moderate to very strong staining of CK13 in the basal layer than those who did not take the medications. In addition, HAART seemed to affect the expression of CK14 and percent of staining cells in the mid/upper prickle cell layer. Significant difference due to HAART status was also observed regarding the expression of CK16 and the inflammation. No significant difference was found with respect to the expression of CK1 and involucrin.

Logistic regression analysis of cytokeratin expression

On logistic regression, the expression of CK13, CK14 and CK16 was found to be significantly different between HIV-infected subjects and non-HIV individuals (Table 4). When using short-term HAART as a reference, a significant difference was noted in the expression of CK14 in the mid/upper prickle cell layer, and CK16 in HIV-infected subjects who were not on HAART. Of interest, the significant difference was also observed in the expression of CK13 in the basal layer of those on long-term HAART (Table 4).

Factors associated with the expression of cytokeratins

Effects of various variables on the expression of CKs are shown in Table 5. The following factors were found to significantly affect the expression of CK14 and CK16: use of HAART, duration of HAART, CD4⁺ cell count, HIV viral load, smoking habit, and alcohol consumption.

Discussion

This study demonstrated that the expression of oral CKs was altered in HIV infection. Although their causal relationships are not known, the expression of CK13, CK14 and CK16, but not CK1 and involucrin, was found to be significantly different between HIV-infected subjects and non-HIV individuals. CK13 and CK14 seemed to be less expressed, while CK16 was more expressed with a weak staining in HIV-infected subjects when compared to non-HIV individuals. The expression of those CKs was also shown to be significantly different between HIV-infected subjects who were on HAART and those who

were not. Long-term use of HAART significantly affected the expression of CK13 in the basal layer among the subjects. The findings of this study suggest that there are gross alterations accruing in CK expression due to HIV infection and HAART.

It is well accepted that HIV infection causes the impairment of both local and systemic immunity (Challacombe and Sweet, 2002). As a consequence, various oral lesions caused by opportunistic infections and malignancies are observed among HIV infected subjects (Laskaris *et al*, 1992; Glick *et al*, 1994; Nittayananta and Chungpanich 1997). Oral epithelial cells, which are part of the local innate immunity (Dale and Fredericks 2005), may also be affected by the infection. However, the mechanisms related to that are not well understood. CKs are the typical intermediate filament proteins of epithelia, which are important for the mechanical stability and integrity of epithelial cells and tissues. The findings of the present study suggest that HIV infection may impair the function of the epithelial cells in innate immunity leading to changes in oral mucosa which is reflected in the alterations in CK expression pattern.

Besides protective function, CKs have evolved to be one of the most potent epithelial differentiation and tumor markers. Specific antibodies against several CKs are routinely used for immunohistochemical typing of carcinomas in tumor diagnostics (Moll *et al*, 2008). Previous studies reported that CKs might serve well to predict malignant transformation of the oral mucosa (Toyoshima *et al*, 2009; Ohkura *et al*, 2005; Schaaïj-Visser *et al*, 2010). For example, CK13 is differentially expressed between normal mucosal epithelium and squamous tumor tissue of the oral cavity (Baker *et al*, 2005; Ohkura *et al*, 2005; Patel *et al*, 2008; Imai *et al*, 2005). The expression of CK13 was shown to be significantly decreased in tumors and severe dysplasia, preneoplastic tissue with a high risk of malignant progression, when compared to normal mucosa (Schaaïj-Visser *et al*, 2009).

CK16 is also typically expressed in OSCC (Moll *et al*, 2008). The induction of CK16 has been reported in various hyperproliferative epidermal disorders, suggesting that this CK may be used as a molecular marker for hyperproliferative keratinocytes (Weiss *et al*, 1984). In addition, CK16 is “stress-inducible” keratin which is rapidly switched on after injury and inflammation (Moll *et al*, 2008).

As the present study demonstrated that the expression of CK13, CK14, and CK16 was significantly different between HIV-infected subjects and non-HIV individuals, these CKs may be used as biomarkers to early detect HIV-infected subjects who are susceptible to malignant transformation of the oral mucosa.

In the present study, changes in the expression of oral CKs were also observed according to HAART status. CK13 was found to be significantly expressed in the basal layer in HIV-infected subjects who were on long-term HAART. As CK13 is usually expressed in the suprabasal compartment in normal buccal mucosa, (Moll *et al*, 2008), this finding may indicate that the pattern of CK13 expression was altered by long-term use of HAART. Of interest, the expression of CK1, which is usually expressed in skin, was also observed on clinically normal buccal mucosa of HIV-infected subjects who were on HAART. This may suggest an early stage of abnormal differentiation of oral epithelial cell due to HAART.

It should be noted that Azidothymidine (AZT, 3'-azido-3'-deoxythymidine, zidovudine) was a mainstay of the NRTI prescribed for the HIV-infected subjects in the present study. Prolonged treatment by AZT potentially causes malignant transformation of oral epithelia (Olivero *et al*, 2007). This may be because AZT is incorporated into DNA causing gene mutations (Olivero *et al*, 2007). In addition, a previous study demonstrated that AZT has genotoxic effects that may lead to genomic instability in cultured cells (Olivero *et al*, 2007). These genetic changes have been used to predict the risk of malignant transformation of oral epithelia (Schaij-Visser *et al*, 2010).

In the HAART era, the incidence of AIDS-defining malignancies (ADMs) including KS and NHL has declined significantly (Simard *et al*, 2010). However, the incidence of other malignancies not known to be associated with immunosuppression (non-ADMs) including OSCC has not changed and remains significantly higher than in the general population (Adler, 2010; Bedimo *et al*, 2008). Potential explanations for the higher non-ADM rates include potential oncogenicity of long-term HIV infection or of long-term HAART (Bedimo *et al*, 2008). Thus, HIV-infected subjects who are on long-term HAART are at risk of developing malignancies including OSCC (Bedimo *et al*, 2008). As the present study demonstrated that the expression of CKs was altered among those who were on HAART, CKs may be the useful biomarkers for monitoring and identifying HIV-infected subjects who are at risk of developing OSCC.

In addition to HIV infection and HAART, the present study demonstrated that various factors known to be associated with the development of oral cancers such as smoking, alcohol, CD4⁺ cell count, and HIV viral load, have also been shown to significantly affect the expression of CK 14 and CK16. It is well accepted that smoking and alcohol consumption have strong causative links to OSCC (White *et al*, 2007). Alcohol serves as a mucosal irritant and may also act as a solvent for the carcinogens found in tobacco. In addition, it may activate members of the cytochrome p450 family enzymes, which is known to activate procarcinogens. This effect of alcohol may be exacerbated when an individual is also exposed to tobacco (Hunter *et al*, 2005).

Infection by human papilloma virus (HPV) is also a risk factor for OSCC that may have synergistic effects with tobacco products (Herrero *et al*, 2003; Mork *et al*, 2001). Our previous study by Amorntharee *et al* (2012) demonstrated that the prevalence of oral HPV type 16 infection was increased in HIV-infected subjects compared to non-HIV individuals. Long-term use of HAART did not seem to decrease a number of the virus in saliva (Amorntharee *et al*, 2012). Further studies should be performed to determine the association between HPV infection and the changes in the pattern of CKs expression that may put the subjects taking HAART at risk of developing OSCC.

The strength of this study was that it used immunohistochemical approaches that could be easily implemented to identify CKs, the protein biomarkers associated with differentiation of epithelial cells (Schaij-Visser *et al*, 2010). Although a causal role in tumorigenesis has not been established for any CKs, changes in the expression of CKs have great importance in immunohistochemical tumor diagnosis of OSCC (Moll *et al*, 2008).

The present study had some limitations. It was conducted as a cross-sectional study. Thus, it lacked the information of changes in the expression of oral CKs overtime in those subjects who were on long-term use of HAART. Longitudinal studies should be performed in the future to demonstrate the effects of long-term use of HAART on the alteration of oral CKs expression. In addition, further studies should be conducted to determine whether the changes are observed in both types of CKs that are co-expressed during the differentiation of the oral epithelial cells.

In conclusion, the present study showed the differential expression of CK13, 14, and CK16 from non-HIV individuals to HIV-infected subjects and to those who were on HAART. The findings suggest that changes in CKs expression did occur in the oral mucosa of HIV-infected subjects who were on long-term HAART. These alterations may have a role in carcinogenesis and have the potential to be used as surrogate markers of malignant transformation. Further studies should be performed in order to gain the insights into the mechanisms how HIV infection and HAART alter the expression of CKs in the oral epithelial cells.

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Figure 1. Changes in oral cytokeratin expression in HIV-infected subjects with long-term use of HAART

Different intensity of cytokeratin 14 staining on the basal cell layer: + weak staining (**a**); ++ moderate staining (**b**); and +++ strong staining (**c**)

Table 1

Antibodies used in the immunohistochemical analysis

Antibody	Manufacturer	Clone	Dilution
Cytokeratin 1	Santa Cruz Biotechnology, Inc.	4D12B3	1:200
Cytokeratin 13	Santa Cruz Biotechnology, Inc.	AE8	1:250
Cytokeratin 14	Santa Cruz Biotechnology, Inc.	LL001	1:100
Cytokeratin 16	Santa Cruz Biotechnology, Inc.	LL025	1:50
Involucrin	Santa Cruz Biotechnology, Inc.	SPM259	1:250

Table 2

Demographic data and characteristics of HIV-infected subjects with and without HAART and non-HIV individuals

Variables	HIV-infected subjects			non-HIV subjects (n=50)
	No HAART (n=58)	with HAART (n=99)		
		Short term HAART (<3 yr) (n=45)	Long term HAART (≥ 3 yr) (n=54)	
Age				
Age range (year)	20–59	23–57	27–53	19–59
Mean age (year)	34	37	40	36
Sex				
Male	20 (34.5%)	18 (40%)	33 (61.1%)	25 (50%)
Female	38 (65.5%)	27 (60%)	21 (38.9%)	25 (50%)
Marital status				
Single	13 (23.6%)	5 (15.2%)	13 (31.7%)	21 (42%)
Married	33 (60.0%)	17 (51.5%)	16 (39.0%)	27 (54%)
Divorce	4 (7.3%)	7 (21.2%)	7 (17.1%)	0 (0%)
Widow	5 (9.1%)	4 (12.1%)	5 (12.2%)	2 (4%)
Highest education				
Primary school level	33 (56.9%)	15 (33.3%)	12 (22.2%)	24 (48%)
Secondary school level	18 (31.0%)	16 (35.6%)	21 (38.9%)	10 (20%)
Polytechnic level	2 (3.4%)	4 (8.9%)	10 (18.5%)	3 (6%)
University level	4 (6.9%)	9 (20.0%)	9 (16.7%)	11 (22%)
Other	1 (1.7%)	1 (2.2%)	2 (3.7%)	2 (4%)
Occupation				
Employee	31 (53.4%)	23 (51.1%)	21 (38.9%)	21 (42%)
Trading	6 (10.3%)	5 (11.1%)	2 (3.7%)	4 (8%)
Agriculture	3 (5.2%)	2 (4.4%)	1 (1.9%)	8 (16%)
Government servant	0 (0.0%)	2 (4.4%)	9 (16.7%)	2 (4%)
Student	1 (1.7%)	0 (0.0%)	0 (0.0%)	8 (16%)
Others	14 (24.1%)	12 (26.7%)	17 (31.5%)	7 (14%)
Unemployed	3 (5.2%)	1 (2.2%)	4 (7.4%)	0 (0%)
Income (Baht)/month				
< 5,000	25 (43.1%)	18 (40.0%)	13 (24.5%)	23 (46%)
5,000–10,000	26 (44.8%)	17 (37.8%)	19 (35.8%)	22 (44%)
10,001–20,000	7 (12.1%)	6 (13.3%)	11 (20.8%)	5 (10%)
20,001–30,000	0 (0.0%)	2 (4.4%)	6 (11.3%)	0 (0%)
> 30,000	0 (0.0%)	2 (4.4%)	4 (7.5%)	0 (0%)

Variables	HIV-infected subjects			non-HIV subjects (n=50)
	No HAART (n=58)	with HAART (n=99)		
		Short term HAART (<3 yr) (n=45)	Long term HAART (≥ 3 yr) (n=54)	
Risk group				
Sex with person with HIV	43 (74.1%)	30 (66.7%)	41 (75.9%)	—
Commercial sex workers	4 (6.9%)	2 (4.4%)	3 (5.6%)	—
MSM	3 (5.2%)	2 (4.4%)	3 (5.6%)	—
IVDU	2 (3.4%)	7 (15.6%)	3 (5.6%)	—
Blood transfusion	1 (1.7%)	0 (0.0%)	1 (1.9%)	—
Other	5 (8.6%)	4 (8.9%)	3 (5.6%)	—
Duration of HIV infection (yr)				
Mean	3.8	4.8	8.8	—
Range	0.1–16	0.4–15	3–24	—
Smoking habit				
Smoker	39 (67.2%)	16 (35.6%)	18 (33.3%)	34 (68%)
Non-smoker	19 (32.8%)	29 (64.4%)	36 (66.7%)	16 (32%)
Alcohol consumption				
Drinker	37 (63.8%)	12 (26.7%)	13 (24.1%)	34 (68%)
Non-drinker	21 (36.2%)	33 (73.3%)	41 (75.9%)	16 (32%)
Presence of HIV-related systemic diseases				
Yes	15 (25.9%)	14 (34.1%)	9 (18.8%)	—
No	43 (74.1%)	27 (65.9%)	39 (81.2%)	—
Oral hygiene				
Good	1 (1.7%)	3 (6.7%)	0 (0.0%)	1 (2%)
Fair	35 (60.3%)	23 (51.1%)	31 (57.4%)	27 (54%)
Poor	22 (37.9%)	19 (42.2%)	23 (42.6%)	22 (44%)
Total lymphocyte cell counts (cell/mm ³)				
<1000	9 (16.7%)	12 (26.7%)	6 (11.1%)	—
1000–2000	22 (40.7%)	14 (31.1%)	14 (25.9%)	—
>2000	23 (42.6%)	19 (42.2%)	34 (63.0%)	—
CD4 ⁺ cell count (cell/mm ³)				
Mean	245.5	250.1	530.7	—
Range	5–669	9–630	74–1,600	—
Viral load (copies)				
Mean	782.6	21,560	5,627	—
Range	0–30,100	50–750,000	50–139,000	—

Table 3
Immunohistochemical staining of oral cytokeratins in HIV-infected subjects with and without HAART and non-HIV individuals

Cytokeratin expression	HIV status		P-value	HAART status of HIV-infected subjects			P-value
	HIV-infected subjects (n= 157)	Non-HIV individuals (n=50)		No HAART (n=58)	Short term HAART (<3 yr) (n=45)	Long term HAART (> 3 yr) (n=54)	
<i>CK13</i>							
Basal							
Weak staining	31 (22.1%)	2 (4.2%)	<0.001	17 (32.1%)	9 (23.1%)	5 (10.4%)	0.02
Moderate staining	73 (52.1%)	25 (52.1%)		22 (41.5%)	21 (53.9%)	30 (62.5%)	
Strong staining	32 (22.9%)	13 (27.1%)		14 (26.4%)	9 (23.1%)	9 (17.6%)	
Very strong staining	4 (2.9%)	8 (16.7%)		0 (0.0%)	0 (0.0%)	4 (8.3%)	
<i>CK14</i>							
Basal							
Absence of staining	0 (0.0%)	0 (0.0%)	<0.001	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.964
Weak staining	20 (13.4%)	5 (10.6%)		8 (14.8%)	5 (11.1%)	7 (14.0%)	
Moderate staining	85 (57.1%)	7 (14.9%)		31 (57.4%)	25 (55.6%)	29 (58.0%)	
Strong staining	44 (29.5%)	35 (74.5%)		15 (27.8)	15 (33.3%)	14 (28.0%)	
Percent of staining cells			0.137				0.774
< 50%	0 (0%)	0 (0.0%)	<0.001	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.087
50–99%	6 (4.0%)	5 (10.6%)		2 (3.7%)	1 (2.2%)	3 (6.0%)	
100%	143 (96.0%)	42 (89.4%)		52 (96.3%)	44 (97.8%)	47 (94.0%)	
Lower prickle							
Absence of staining	94 (64.0%)	14 (29.8%)	<0.001	29 (55.8%)	31 (68.9%)	34 (68.0%)	0.087
Weak staining	41 (27.9%)	26 (55.3%)		21 (40.4%)	8 (17.8%)	12 (24.0%)	
Moderate staining	12 (8.2%)	7 (14.9%)		2 (3.9%)	6 (13.3%)	4 (8.0%)	
Strong staining	0 (0.0%)	0 (0.0%)	0.138	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.739
Percent of staining cells							
< 20%	109 (74.2%)	25 (53.2%)		36 (69.2%)	33 (73.3%)	40 (80.0%)	
20–49%	24 (16.3%)	18 (38.3%)		10 (19.2%)	7 (15.6%)	7 (14.0%)	
50–79%	8 (5.4%)	3 (6.4%)		3 (5.8%)	4 (8.9%)	1 (2.0%)	

Cytokeratin expression	HIV status		P-value	HAART status of HIV-infected subjects			P-value
	HIV-infected subjects (n= 157)	Non-HIV individuals (n=50)		No HAART (n=58)	Short term HAART (<3 yr) (n=45)	Long term HAART (> 3 yr) (n=54)	
>80%	6 (4.1%)	1 (2.1%)	<0.001	3 (5.8%)	1 (2.2%)	2 (4.0%)	<0.001
Mid/Upper prickle							
Absence of staining	125 (85.0%)	28 (59.6%)		38 (73.1%)	45 (100.0%)	42 (84.0%)	
Weak staining	20 (13.6%)	9 (19.2%)		14 (26.9%)	0 (0.0%)	6 (12.0%)	
Moderate staining	0 (0.0%)	9 (19.2%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	
Strong staining	2 (1.4%)	1 (2.1%)	0.828	0 (0.0%)	0 (0.0%)	2 (4.0%)	0.001
Percent of staining cells							
< 20%	132 (89.8%)	42 (89.4%)		40 (76.9%)	45 (100.0%)	47 (94.0%)	
20–49%	11 (7.5%)	4 (8.5%)		9 (17.3%)	0 (0.0%)	2 (4.0%)	
50–79%	2 (1.4%)	1 (2.1%)		2 (3.9%)	0 (0.0%)	0 (0.0%)	
>80%	2 (1.4%)	0 (0%)		1 (1.9%)	0 (0.0%)	1 (2.0%)	
CK16							
Absence of staining	61 (42.7%)	36 (76.6%)	<0.001	13 (24.5%)	13 (33.3%)	35 (68.6%)	<0.001
Weak staining	72 (50.4%)	11 (23.4%)		35 (66.0%)	22 (56.4%)	15 (29.4%)	
Moderate staining	10 (7.0%)	0 (0.0%)		5 (9.4%)	4 (10.3%)	1 (2.0)	
Strong staining	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	
Percent of staining cells							
< 10%	89 (62.2%)	44 (93.6%)	<0.001	20 (37.7%)	31 (79.5%)	38 (74.5%)	<0.001
10–29%	40 (28.0%)	3 (6.4%)		21 (39.6%)	8 (20.5%)	11 (21.6%)	
30–50%	11 (7.8%)	0 (0.0%)		10 (18.9%)	0 (0.0%)	1 (2.0%)	
> 50%	3 (2.1%)	0 (0.0%)		2 (2.8%)	0 (0.0%)	1 (2.0%)	

Note: The percent of staining is not shown for CK13 because all target cells in the suprabasal layer were positive staining.

Table 4

Effects of HIV and HAART status on the expression of oral cytokeratins based on logistic regression

Types of cytokeratin	Adjusted odd ratios ^a (95% Confidence Interval)			
	HIV status		HAART status ^b	
	HIV-negative	HIV-positive	No HAART	Long-term use of HAART
CK1 expression (ref. <20%)	1.00	0.77 (0.39, 1.49)	0.85 (0.37, 1.97)	0.78 (0.34, 1.76)
CK13 expression (ref. = weak staining)	1.00	0.17 (0.04, 0.76)*	2.71 (0.86, 8.50)	4.06 (1.36, 12.10)*
CK14 expression				
basal (ref. <50%)	1.00	4.24 (1.14, 15.69)*	0.29 (0.03, 1.96)	0.36 (0.03, 3.55)
lower (ref. <20%)	1.00	0.38 (0.19, 0.75)*	0.49 (0.19, 1.25)	0.69 (0.26, 1.79)
mid (ref. <20%)	1.00	0.61 (0.19, 1.96)	0.17 (0.04, 0.68)*	6.70 (0.62, 911.2)
CK16 expression (ref. = weak staining)	1.00	7.48 (2.13, 26.30)*	0.11 (0.04, 0.33)*	1.17 (0.42, 3.26)
Involucrin (ref. absent or mild)	1.00	0.82 (0.37, 1.81)	0.99 (0.41, 2.40)	0.78 (0.30, 2.06)
Degree of inflammation (ref. absent or mild)	1.00	1.26 (0.14, 11.61)	0.31 (0.03, 1.85)	2.88 (0.14, 432.2)

^a After controlling for duration of HIV infection, CD4 cell counts, smoking habits, and alcohol consumption.^b Short-term HAART was used as a reference

* P-value<.05

Table 5
Effects of long-term use of HAART and other variables on the expression of oral cytokeratins in HIV-infected subjects

Predictors for cytokeratin expression	Difference of mean percent positive cells between groups											
	CK1		CK13		CK14 basal		CK14 lower		CK14 mid		CK16	
	diff. mean (%)	P-value *	diff. mean (%)	P-value *	diff. mean (%)	P-value *	diff. mean (%)	P-value *	diff. mean (%)	P-value *	diff. mean (%)	P-value *
HIV status positive vs negative	-2.05	0.505	-0.50	0.916	1.48	0.864	-6.99	0.611	-1.78	0.748	6.76	0.443
Duration of HIV infection 5–10 vs < 5 years	-3.43	0.331	0.15	0.357	-3.43	0.727	-1.22	0.572	-2.10	0.565	-2.69	0.159
HAART status yes vs no	4.39	0.193	0.35	0.086	0.34	0.206	-1.50	0.074	-5.23	<0.001	-11.39	<0.001
Duration of HAART long vs short term	-7.71	0.074	0.25	0.127	-1.58	0.281	-1.49	0.518	3.90	0.028	0.46	0.955
CD4 ⁺ cell count <200 vs ≥ 200 cell/cm ³	4.46	0.286	0.08	0.488	-1.03	0.341	-1.03	0.337	-1.86	0.285	-7.84	<0.001
Viral load ≥ 50 vs < 50	3.99	0.411	0.24	0.050	-1.25	0.223	-4.26	0.005	-5.22	<0.001	-8.83	<0.001
Smoking Yes vs No	-1.38	0.730	-0.01	0.737	-0.86	0.664	-6.12	0.452	-1.17	0.905	4.40	0.004
Alcohol Yes vs No	-3.32	0.262	-0.15	0.521	-0.11	0.217	-5.98	0.340	-0.63	0.983	4.75	0.013

* P-value from Anova test, adjusted for other factors