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Expression of oral cytokines in HIV-infected subjects with long-term use of antiretroviral therapy

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Abstract

Objectives—The objectives of this study were to determine 1) the expression of oral pro-inflammatory cytokines in HIV-infected subjects compared with non-HIV individuals, 2) the cytokine expression in the subjects with antiretroviral therapy (ART) compared with those without ART, and 3) factors associated with the expression of the cytokines.

Materials and methods—Oral examination was performed and saliva samples were collected and analyzed for the expression of pro-inflammatory cytokines using ELISA. Logistic regression analysis was performed to determine the association between HIV/ART status and the cytokine expression.

Results—One hundred and fifty-seven HIV-infected subjects with and without ART, and 50 non-HIV individuals were enrolled. TNF-α and IL-6 in saliva were significantly decreased, while IL-8 was significantly increased in HIV infection ($p < 0.05$). Changes in the expression of IL-8 was also observed between HIV-infected subjects who were and were not on ART ($p < 0.05$). Duration of HIV infection and smoking were significantly associated with the expression of pro-inflammatory cytokines in saliva ($p < 0.05$).

Conclusion—Oral innate immunity is affected by HIV infection and use of ART. IL-8 may be the useful biomarker to identify subjects at risk of infection and malignant transformation due to HIV infection and long-term use of ART.

Keywords

antiretroviral therapy; cytokines; ART; HIV; pro-inflammatory cytokines; saliva

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Author contributions

Prof. Dr. Wipawee Nittayananta developed the proposal, applied for the research grant, directed the project, interpreted the results, prepared and revised the manuscript; Ms. Korntip Amornthatree and Ms. Marisa Kemapunmanus performed ELISA to detect cytokines in saliva, Dr. Sineepat Talungchit examined patients and collected saliva samples; Assoc. Prof. Dr. Hutcha Sriplung contributed to statistical analysis.

Introduction

Infection with human immunodeficiency virus (HIV) appears to have both direct and indirect effects on systemic and local innate immunity leading to the development of oral opportunistic infections and malignancies (Challacombe and Naglik, 2006). Antiretroviral therapy (ART) is the standard treatment of HIV infection, which consists of a combination of three or four drug groups including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INIs), and fusion inhibitors (FIs) (Hammer *et al*, 2008).

It is well accepted that ART contributed to a global reduction of HIV-associated oral lesions (Patton *et al*, 2000; Nokta 2008; Miziara *et al*, 2008). However, it has been reported that prolonged treatment by Azidothymidine (AZT, 3′-azido-3′-deoxythymidine, zidovudine) a mainstay of the NRTI used among HIV-infected subjects, potentially causes malignant transformation of oral epithelia (Olivero 2007). Long-term use of this medication may contribute to an increased risk for non-AIDS related malignancies of different organs including oropharynx that has been observed among HIV-infected subjects even in the ART era (Clifford *et al*, 2005; Gillison 2009).

Cytokines play a significant role in regulating the differentiation, proliferation, and function of mammalian cells (Dongari-Bagtzoglou and Fidel 2005). Oral epithelial cells are among the main sources of the cytokines in saliva (Dongari-Bagtzoglou and Fidel 2005). During oral infection, a large number of pro-inflammatory and immunoregulatory cytokines are generated. Certain pro-inflammatory cytokines appear to play a regulatory role in the direct antimicrobial activity of oral epithelial cells (Dongari-Bagtzoglou and Fidel 2005). Pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 8 (IL-8) have been shown to exert various biological functions including regulating inflammatory response and cancer development (Brailo *et al*, 2012). These molecules maintain a central role in the host protective immunity. Thus, changes in the expression of these pro-inflammatory cytokines may dictate the host defense processes.

Because HIV infection can promote the development of malignancy (Clifford *et al*, 2005), and long-term use of ART has the potential oncogenicity (Bedimo *et al*, 2008), it is important to determine if HIV infection and long-term use of ART adversely affect the local innate immunity. The objectives of this study were to determine 1) the expression of pro-inflammatory cytokines in saliva of HIV-infected subjects compared with non-HIV individuals, 2) the oral pro-inflammatory cytokine expression in the subjects with ART compared with those without ART, and 3) factors associated with the expression of salivary pro-inflammatory cytokines.

Materials and methods

Subjects

A cross-sectional study was performed in HIV-infected subjects who came to receive ART at the Internal Medicine Clinic at Songklanagarind Hospital and Hat Yai Regional Hospital in southern Thailand. The inclusion criteria of subjects enrolled as a study group 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 ART, and iii) willing to participate in the study. Severely ill HIV-infected subjects who could not cooperate with the procedures of saliva collection were excluded. A group of HIV-infected individuals who came to those hospitals

but had not yet started ART and a group of non-HIV infected volunteer with the same age range as those in the study group 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/en/20activities/10ethics/10helsinki/index.html). The study protocol was approved by the research committee at the Prince of Songkla University, and at the Ministry of Public Health. All information about the patients and their identity were anonymous. Subjects were given both verbal and written information about the nature of the study and written consent obtained. 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). Periodontal health and oral hygiene were assessed using criteria as previously described (Silness and Loe 1964). The following data were recorded; HIV status, duration of HIV infection (calculated from the time since HIV seropositivity was first diagnosed), use of ART, duration of ART, $CD4^+$ cell count, HIV viral load, smoking habit and alcohol consumption.

Measurement of salivary flow rate and saliva collection

Measurement of salivary flow rate and collection of saliva were conducted only in the morning between 9:00 a.m.–12:00 a.m. to minimize variation effects. Participants were refrained from eating and tooth-brushing for at least 30 min before saliva collection. All subjects were asked to rinse their mouth with water and spit out, and thereafter swallow before starting the collection procedure comprising of both unstimulated whole saliva using the draining technique and wax-stimulated whole saliva as previously described (Nittayananta *et al*, 2010). Saliva samples were kept at −80 °C within 2 h of collection. Samples were later thawed, mixed briefly, and analyzed for TNF-α, IL-6 and IL-8 contents using ELISA (PeproTech, Rohovot, Israel).

Quantitation of salivary pro-inflammatory cytokine levels

The levels of TNF-α, IL-6 and IL-8 in both unstimulated and stimulated saliva were quantified by ELISA based on matched anti-TNF-α, anti-IL-6, and anti-IL-8 (PeproTech, Rohovot, Israel). Recombinant TNF-α, IL-6, and IL-8 were used as standards (PeproTech, Rohovot, Israel). Optical density measurements were performed using the Behring ELISA Processor III (Dade Behring Diagnostic Co Ltd., Deerfield, IL, USA). All samples were run in duplicate diluted 1:2.

Statistical analysis

Descriptive statistics were used to analyze breakdown of subjects by status of HIV test and duration of ART received. Appropriate tests according to the type and distribution of the data of interest were employed to explore possible association between salivary pro-inflammatory cytokine levels and HIV/ART status. Finally, as the expression of oral cytokines may be influenced by duration of HIV infection, $CD4^+$ cell count, smoking and drinking behaviors, multiple logistic regression within the same condition of HIV/ART status was used to analyze the relationship between salivary pro-inflammatory cytokine levels with HIV status and duration of ART use, respectively, so that the effect of potential confounding factors can be controlled. To accommodate the non-normal distribution of the

data, Kruskal-Wallis test was used to see the effect of various predictors on the expression of TNF-α, IL-6, and IL-8, respectively. Statistical significance was set at 0.05.

Results

Subjects and use of ART

Ninety nine HIV-infected subjects receiving ART (age range 23–57 yr, mean age 39 yr), 58 receiving no ART (age range 20–59 yr, mean age 34 yr), and 50 non-HIV individuals (age range 19–59 yr, mean age 36 yr) were enrolled. All of them were Thai. Most HIV-infected subjects who were on ART received no PI based regimen (n=84, 85%). Different combinations of ART 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 ART, and those who had been taking ART for ≥3 years were classified as a group with long-term use of ART, respectively. Various characteristics of the subjects enrolled are shown in Table 1.

Oral health status and salivary flow rates in HIV-infected subjects with and without ART and non-HIV individuals

HIV-infected subjects were presented with a significantly higher prevalence of oral lesions than non-HIV individuals (Chi-square test, $p<0.001$) (Table 2). The significant difference was also observed with respect to their ART status. Hyperpigmentation was the most common oral lesions followed by denture stomatitis. Oral candidiasis and oral hairy leukoplakia, the two most common oral lesions seen among HIV-infected subjects before the ART era, were diagnosed in only two and one subjects who received ART, respectively. Of interest, no oral warts were diagnosed among the subjects. Presence of periodontal pocket depths ≥ 4 mm and bleeding on probing in HIV-infected subjects were significantly associated with the ART status. Salivary flow rates of both unstimulated and stimulated saliva subjects were statistically significant lower in HIV-infected than non- HIV individuals (Chi-square test, $p<0.001$). Unstimulated salivary flow rates were significantly associated with the ART status of the subjects.

Expression of pro-inflammatory cytokines in saliva of HIV-infected subjects with and without ART and non-HIV individuals

The expression of TNF-α and IL-6 was significantly decreased in HIV-infected subjects compared with non-HIV individuals ($p <0.05$) (Table 3). In contrast, IL-8 was significantly increased in HIV infection ($p< 0.05$). According to the ART status, the expression of TNF-α and IL-6 were not significantly different between those who were and were not on the medication. The expression of IL-8 in stimulated saliva, however, was significantly increased with the use of ART. Nevertheless, the expression seemed to be decreased with long-term use of the medication (Table 3).

Logistic regression analysis of the expression of pro-inflammatory cytokines in saliva

On logistic regression models for the three salivary cytokine outcomes under unstimulated and stimulated conditions, HIV and ART statuses adjusted for other potential confounding factors (i.e. duration of HIV infection, $CD4^+$ cell count, HIV viral load, oral health status, smoking and drinking behaviors), the expression of IL-8 in stimulated saliva was significantly different in HIV-infected subjects compared with non-HIV individuals (Table 4). Significant changes in the expression of IL-6 in unstimulated saliva were found in those with long-term use of ART. However, no significant difference in the expression of TNF-α was observed with respect to HIV infection and ART status of the subjects.

Factors associated with the expression of pro-inflammatory cytokines in saliva

Effects of various variables on the expression of pro-inflammatory cytokines in saliva are shown in Table 5. Duration of HIV infection was found to significantly affect the expression of TNF- α and IL-8, while smoking was found to influence the expression of IL-6.

Discussion

This study demonstrated that salivary pro-inflammatory cytokines, which are parts of oral innate immunity, were altered by HIV infection and use of ART. Although their causal relationships are not known, the expression of both TNF-α and IL-6 in saliva were significantly decreased but IL-8 was significantly increased in HIV infection. Salivary levels of IL-8 were also found to be significantly different between HIV-infected subjects who were and were not on ART.

In the present study, differences were observed in the cytokine profiles in saliva of HIV-infected subjects compared with non-HIV infected individuals, and among HIV-infected subjects who were on long-term ART compared with those who were not. These findings suggest that the local immune system is affected by HIV infection and long-term use of ART. The local innate immunity in HIV-infected subjects may also be influenced by the presence of some opportunistic infections resulting in the changes of salivary cytokine profiles among the subjects (Black *et al*, 2000). However, it is possible that an aberrant expression of some cytokines may have predisposed those subjects to opportunistic infections and malignancies in the oral cavity.

The impairment of both local and systemic immunity is noted in HIV infection (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). A previous study reported that opportunistic infections by oral *Candida* and Epstein-Barr virus may influence the local innate immunity in HIV-infected subjects resulting in the changes of salivary cytokine profiles in those with oral candidiasis and oral hairy leukoplakia (Black *et al*, 2000). However, these two HIV-related oral lesions were rarely seen in the present study. Thus, it was not possible to correlate the changes in salivary pro-inflammatory cytokine levels with the presence of those oral lesions.

In this study, TNF-α was significantly decreased in HIV-infected subjects compared with non-HIV individuals. This finding is consistent with that of the previous study (Black *et al*, 2000) and may indicate that HIV infection has a significant effect on the local production of cytokines in the oral cavity. However, a previous study reported that elevated levels of TNF-α was observed in the sera of HIV-infected subjects (Lähdevirta *et al*, 1988). This may suggest the dichotomy between local and systemic immunity (Sweet *et al*, 1995). As TNF-α can be inhibited by other cytokines such as TGF-β and IL-4, the decreased expression of TNF-α in HIV-infected subjects may also be explained by an interplay among the cytokines (Black *et al*, 2000). In addition, it has been demonstrated that regulation of TNF-α synthesis by any specific cells depends on the distribution of TNF-α receptors as well as the differential use of regulatory elements of TNF-α biosynthesis in different cells (Zhang and Tracey, 1998).

In the present study, significant changes in the expression of IL-8 in stimulated saliva were observed with respect to ART status (Table 3). These findings may indicate that use of ART affects the local innate immunity of HIV-infected subjects. However, no significant differences in the expression of TNF-α and IL-6 were noted according to their ART status.

Increased expression of the pro-inflammatory cytokines in saliva has been observed in patients with oral leukoplakia and oral cancers (Brailo *et al*, 2006; Brailo *et al*, 2012; Vairaktaris *et al*, 2008). It should be noted that AZT 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 (Schaaij-Visser *et al*, 2010).

In ART era, the incidence of AIDS-defining malignancies (ADMs) including Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL) has declined significantly (Sigmard *et al*, 2010). However, the incidence of other malignancies not known to be associated with immunosuppression (non-ADMs) including oral squamous cell carcinoma (OSCC) remains significantly higher than in the general population (Bedimo *et al*, 2008; Adler *et al*, 2010). A study by McLemore et al (2010) reported that OSCC was diagnosed in 4 out of 40 (10%) HIV-infected patients with head and neck squamous cell carcinoma. The higher non-ADM rates may be due to potential oncogenicity of long-term HIV infection or of long-term ART (Bedimo *et al*, 2008). Thus, HIV-infected subjects who are on long-term ART are at risk of developing malignancies including OSCC (Bedimo *et al*, 2008). As the present study demonstrated that levels of IL-8 in stimulated saliva were significantly altered among those who were on ART, this salivary pro-inflammatory cytokine may be the useful biomarker for monitoring and identifying HIV-infected subjects who are at risk of developing OSCC.

It is well accepted that cytokines act locally and play a crucial role in mucosal innate immunity (Nicol *et al*, 2008). Thus, examining their concentrations in specific organs, such as the oral cavity is important. Since TNF-α is a key Toll-like receptor (TLR)-induced pro-inflammatory cytokine that has pleiotropic biological actions (Nicol *et al*, 2008), changes in its salivary levels may help explain the increased occurrence of oral lesions in HIV-infected subjects on long-term ART. Although our study could not demonstrate significant changes of TNF-α in patients on long-term ART, further studies should be performed to determine if the expression of TLRs is altered by HIV infection and long-term ART with subsequent changes in mitogen-activated protein kinase signaling and cytokine production. These may ultimately lead to deficiencies of innate immune responses that predispose HIV-infected subjects to infection and malignant transformation.

In the present study, various factors known to be associated with the development of oral cancers including duration of HIV infection and smoking have been shown to significantly affect the expression of pro-inflammatory cytokines in saliva. It is well accepted that smoking has strong causative links to OSCC (White *et al*, 2007). In addition, infection by human papilloma virus (HPV), another risk factor for OSCC, may have synergistic effects with tobacco products (Mork *et al*, 2001; Herrero *et al*, 2003). Our previous study demonstrated that the prevalence of oral HPV type 16 (HPV-16) infection was increased in HIV-infected subjects compared with non-HIV individuals, and long-term use of ART did not seem to decrease a number of the virus in saliva (Amornthatree *et al*, 2012). Further studies should be performed to determine the association between HPV-16 infection and the changes in the expression of pro-inflammatory cytokines in saliva that may put HIV-infected subjects on long-term ART at risk for developing OSCC.

The strength of this study was that it focused on the expression of salivary pro-inflammatory cytokines, which are the protein biomarkers associated with different oral infections and cancers (Dongari and Fidel 2005; Vairaktaris *et al*, 2008). Although a causal role in tumorigenesis has not been established for TNF-α, IL-6 and IL-8, changes in the levels of

these pro-inflammatory cytokines seem to have great importance as biomarkers of OSCC (Vairaktaris *et al*, 2008).

This study had some limitations. First, the presence of tooth decay, which may affect the expression of oral cytokines, was not recorded. Second, it was conducted as a cross-sectional study. Thus, it lacked the information of changes in the expression of oral pro-inflammatory cytokines overtime in those subjects who were on long-term use of ART. Longitudinal studies should be performed in the future to better demonstrate the effects of long-term use of ART on the alteration of salivary pro-inflammatory cytokine levels. In addition, further studies should be conducted to assess the changes in other types of cytokines that may also involve in malignant transformation of the oral mucosa.

In conclusion, the present study demonstrated the changes in the expression of salivary pro-inflammatory cytokines with HIV infection. In addition, the levels of IL-8 in stimulated saliva were significantly altered by ART status of HIV-infected subjects. The findings suggest that changes in the production of IL-8 did occur in the oral cavity of the subjects who were on long-term ART. 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 ART alter the expression of IL-8 in saliva.

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Table 1

Demographic data and characteristics of HIV-infected subjects with and without antiretroviral therapy (ART) and non-HIV individuals

Variables	HIV-infected subjects			non-HIV subjects (n=50)
	No ART (n=58)	with ART (n=99)		
		Short term ART (<3 yr) (n=45)	Long term ART (≥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 (50.0%)
Female	38 (65.5%)	27 (60%)	21 (38.9%)	25 (50.0%)
Duration of HIV infection (yr)				
Mean	3.8	4.8	8.8	-
Range	0.1–16	0.4–15	3–24	-
Presence of HIV-related systemic diseases	15 (25.9%)	14 (34.1%)	9 (18.8%)	-
Smoking habit				
Smoker	39 (67.2%)	16 (35.6%)	18 (33.3%)	34 (68.0%)
Non-smoker	19 (32.8%)	29 (64.4%)	36 (66.7%)	16 (32.0%)
Alcohol consumption				
Drinker	37 (63.8%)	12 (26.7%)	13 (24.1%)	34 (68.0%)
Non-drinker	21 (36.2%)	33 (73.3%)	41 (75.9%)	16 (32.0%)
Oral hygiene				
Good	1 (1.7%)	3 (6.7%)	0 (0.0%)	1 (2.0%)
Fair	35 (60.3%)	23 (51.1%)	31 (57.4%)	27 (54.0%)
Poor	22 (37.9%)	19 (42.2%)	23 (42.6%)	22 (44.0%)
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	-
HIV viral load (copies/mm^3)				
Mean	782.6	21,560	5,627	-
Range	0–30,100	50–750,000	50–139,000	-

Table 2

Oral health parameters, salivary flow rates in HIV-infected subjects with and without antiretroviral therapy (ART) and non-HIV individuals

Variables	HIV status			ART status of HIV-infected subjects			
	HIV-infected subjects (n= 157)	Non-HIV individuals (n=50)	*P*-value	No ART (n=58)	Short-term ART (<3 yr) (n=45)	Long-term ART (≥3 yr) (n=54)	*P*-value
Presence of oral lesions	110 (70%)	21 (42%)	< 0.001	46 (81%)	26 (57%)	38 (70%)	0.029
Presence of periodontal pockets≥4 mm	127 (82%)	41 (85%)	0.734	51 (89%)	31 (69%)	45 (85%)	0.022
Presence of bleeding on probing	146 (94%)	46 (96%)	0.736	57 (100%)	38 (83%)	51 (96%)	< 0.001
Salivary flow rates (ml/min)							
- Unstimulated saliva							
Range	0.1–0.4	0.2–0.6	< 0.001	0.1–0.4	0.1–0.3	0.1–0.4	0.026
Median	0.2	0.4		0.2	0.2	0.3	
- Stimulated saliva							
Range	0.9–2.2	1.4–2.7	< 0.001	0.9–2.3	0.7–2.1	1.2–2.4	0.089
Median	1.5	1.9		1.3	1.4	1.7	

Table 3

Expression of TNF-α, IL-6, IL-8 in saliva of HIV infected subjects with and without antiretroviral therapy (ART) and non-HIV individuals

Median (Q1,Q3,max.) of pro-inflammatory cytokine levels in saliva (ng/ml)	HIV status		P-value[1]	ART status of HIV-infected subjects			P-value[2]
	HIV-infected subjects (n=157)	Non-HIV individuals (n=50)		No ART (n=58)	Short-term ART (<3 yr) (n=45)	Long- term ART (≥3 yr) (n=54)	
TNF-α							
- unstimulated saliva	16.2 (0.0, 114.1, 316.0)	121.5 (0.0, 188.7, 280.0)	0.002	25.4 (0.0, 109.5, 255.0)	0.0 (0.0, 86.0, 234.3)	41.0 (0.0, 133.2, 316.0)	0.348
- stimulated saliva	0.0 (0.0, 0.0, 35.2)	0.0 (0.0, 0.0, 110.8)	0.007	0.0 (0.0, 0.0, 33.3)	0.0 (0.0, 0.0, 35.2)	0.0 (0.0, 0.0, 0.0)	0.120
IL-6							
- unstimulated saliva	164.3 (0.0, 294.4, 431.6)	197.9 (0.0, 268.4, 444.5)	0.837	43.7 (0.0, 289.8, 431.6)	233.1 (29.2, 294.4, 357.9)	182.2 (47.4, 301.5, 414.8)	0.180
- stimulated saliva	0.0 (0.0, 0.0, 174.3)	0.0 (0.0, 21.8, 358.1)	0.003	0.0 (0.0, 0.0, 109.7)	0.0 (0.0, 0.0, 174.3)	0.0 (0.0, 0.0, 164.9)	0.648
IL-8							
- unstimulated saliva	110.8 (72.6, 177.4, 886.9)	106.5 (54.3, 134.6, 226.8)	0.089	104.6 (50.5, 162.5, 440.5)	87.1 (72.6, 169.2, 430.5)	140.5 (97.8, 197.0, 886.9)	0.060
- stimulated saliva	72.3 (0.0, 188.0, 710.3)	0.0 (0.0, 73.2, 559.8)	0.003	21.5 (0.0, 181.6, 481.2)	90.0 (39.6, 172.1, 710.3)	72.3 (0.0, 201.7, 644.1)	0.043

[1] Wilcoxon test

[2] Kruskal Wallis test

Table 4

Effects of HIV status and long-term use of antiretroviral therapy (ART) on the expression of oral pro-inflammatory cytokines based on logistic regression

Pro-inflammatory cytokines	Adjusted odd ratios* (95% Confidence Interval)		
	HIV status	ART status	
	HIV-positive (HIV-negative as reference)	Short-term ART (No ART as reference)	Long-term ART (No ART as reference)
TNF-α			
TNF-α level in saliva >10 ng/ml			
-unstimulated	0.45 (0.21, 0.97)	0.85 (0.38, 1.90)	1.18 (0.53, 2.64)
-stimulated	0.30 (0.07, 1.26)	3.00 (0.16, 441.76)	0.14 (0.001, 3.22)
IL-6			
IL-6 level in saliva >10 ng/ml			
-unstimulated	0.87 (0.39, 1.93)	0.27 (0.10, 0.72)	3.57 (1.38, 9.23)
-stimulated	0.23 (0.07, 0.74)	0.48 (0.05, 2.87)	2.77 (0.29, 26.86)
IL-8			
IL-8 level in saliva >70 ng/ml			
-unstimulated	1.35 (0.64, 2.86)	0.39 (0.14, 1.05)	2.57 (0.95, 6.94)
-stimulated	2.31 (1.09, 4.93)	0.61 (0.28, 1.32)	1.54 (0.71, 3.36)

*Odds ratios for 3 pro-inflammatory cytokine outcomes under 2 conditions (unstimulated *vs* stimulated) and 3 HIV-ART statuses were adjusted for other potential confounding factors (not shown on the table).

Table 5

Effects of long-term use of antiretroviral therapy (ART) and other variables on the expression of oral pro-inflammatory cytokines in HIV-infected subjects

Predictors for the expression	Median (Q1, Q3) values between groups					
	TNF-α	P-value*	IL-6	P-value*	IL-8	P-value*
Duration of HIV infection		0.015		0.055		0.030
< 5 years	55.0 (0.0, 193.0)		127.0 (0.0, 288.4)		109.7 (65.2, 180.0)	
5–10 years	0.0 (0.0, 87.6)		221.7 (78.3, 310.3)		132.6 (97.8, 185.2)	
Duration of ART use		0.233		0.150		0.079
No ART	25.4 (0.0, 109.5)		43.7 (0.0, 289.8)		104.6 (50.5, 162.5)	
Short-term ART	0.0 (0.0, 86.0)		233.1 (29.2, 294.4)		87.1 (72.6, 169.2)	
Long-term ART	41.0 (0,0, 133.2)		182.2 (47.4, 301.5)		140.5 (97.8, 197.0)	
$CD4^+$ cell count		0.683		0.122		0.631
<200 cell/mm^3	10.8 (0.0, 153.7)		225.7 (0.0, 314.0)		100.4 (50.2, 168.2)	
≥200 cell/mm^3	18.0 (0.0, 99.6)		135.6 (0.0, 287.7)		113.1 (79.4, 173.6)	
HIV viral load		0.548		0.069		0.198
< 50 (copies/mm^3)	14.3 (0.0, 108.0)		47.9 (0.0, 288.7)		109.0 (53.6, 173.6)	
≥ 50 (copies/mm^3)	27.7 (0.0, 126.1)		188.9 (38.2, 310.7)		117.5 (75.2, 179.1)	
Smoking		0.057		0.015		0.442
Yes	70.0 (0.0, 188.3)		253.8 (72.5, 320.6)		118.8 (83.2, 152.5)	
No	21.5 (0.0, 105.3)		101.6 (0.0, 269.1)		104.5 (60.8, 169.9)	
Alcohol		0.303		0.058		0.893
Yes	61.8 (0.0, 183.2)		224.5 (75.6, 324.8)		113.1 (71.1, 152.2)	
No	19.0 (0.0, 121.1)		105.0 (0.0, 278.5)		110.8 (62.9, 168.3)	

*P-value from Kruskal-Wallis test, adjusted for other factors