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Oral human β-defensin 2 in HIV-infected subjects with long-term use of antiretroviral therapy

Wipawee Nittayananta[1], **Marisa Kemapunmanus**[2], **Kornthip Amornthatree**[2], **Sineepat Talungchit**[3], and **Hutcha Sriplung**[1]

[1]Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand [2]Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand [3]Department of Oral Surgery and Oral Medicine, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand

Abstract

BACKGROUND—The objectives of this study were to determine 1) oral hBD2 expression in HIV-infected subjects compared to non-HIV controls, 2) the expression of oral hBD2 in HIV-infected subjects with ART compared with those without ART, and 3) factors associated with the expression of oral hBD2.

METHODS—Oral examination and punched biopsy on buccal mucosa were performed in HIV-infected subjects with and without ART, and non-HIV individuals. The expression of hBD2 mRNA was determined by quantitative real-time PCR. Saliva samples of both un-stimulated and stimulated saliva were collected and analyzed for hBD2 levels using ELISA. Student's t-test and nonparametric multi-way ANOVA test were used for comparison of measurements between or among groups.

RESULTS—One hundred and fifty-seven HIV-infected subjects were enrolled; 99 on ART (age range 23–57 yr, mean 39 yr), 58 not on ART (age range 20–59 yr, mean 34 yr), and 50 non-HIV controls (age range 19–59 yr, mean 36 yr). The most common ART regimen was 2 NRTIs+1 NNRTI. Salivary levels of hBD2 were significantly increased in HIV infection ($p< 0.001$). The levels of hBD2 in stimulated saliva were also found to be significantly different between HIV-infected subjects who were and were not on ART ($p< 0.001$). No significant difference was observed with the expression of hBD2 mRNA.

CONCLUSION—Oral innate immunity is affected by HIV infection and use of ART. Salivary hBD2 levels may be the useful biomarkers to monitor those on long-term ART who are at risk of developing oral infections and malignant transformation.

Keywords

ART; defensins; hBD2; HIV; oral health; oral lesion; risk factor; saliva

Introduction

HIV infection appears to have both direct and indirect effects on systemic and local innate immunity leading to the development of oral opportunistic infections and malignancies (1). Antiretroviral therapy (ART) is the standard treatment of HIV-infection, which consists of a

Corresponding address: Prof. Dr. Wipawee Nittayananta, Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand, Fax: 66-74-212-900, wipawee.ni@psu.ac.th.

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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) (2). It is well accepted that ART contributed to a global reduction of HIV-associated oral lesions (3–5).

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 (6). 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 (7, 8). Thus, it is important to determine if HIV infection and long-term use of ART adversely affects the local innate immune response that put the subjects at risk of developing oral infections and cancers.

Human β-defensins (hBDs) produced by oral epithelial cells act as part of the innate antimicrobial defense (9). They are expressed in buccal mucosa, gingiva, tongue, salivary glands and other oral regions (10–12). HBDs exhibit broad antimicrobial activities and are believed to be involved in the maintenance of homeostasis in the oral cavity (13, 14). Previous studies reported altered expression of hBDs in oral squamous cell carcinoma (OSCC) suggesting their involvement in carcinogenesis (14, 15) and indicate that hBDs may be useful markers of OSCC.

Thus, the objectives of this study were to determine 1) the expression of hBD2 mRNA and its salivary protein levels in HIV-infected subjects compared with non-HIV controls, 2) the expression of hBD2 mRNA and its salivary protein levels in HIV-infected subjects with ART compared with those without ART, and 3) factors associated with the expression of hBD2 mRNA and its salivary protein levels.

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 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. The exclusion criteria were i) severely ill HIV-infected subjects who could not cooperate with the procedures of tissue and saliva samples collection, 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 ART, and non-HIV infected volunteer were asked to participate as controls.

Ethics

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.

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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 criteria classified by the EC-Clearinghouse (16). The following data were recorded; HIV status, duration of HIV infection, use of ART, duration of ART, $CD4^+$ 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 buccal mucosa of all HIV-infected subjects and non-HIV controls. Immediately after collection, specimen was placed in RNA later (Qiagen Inc., Valencia, CA, USA) and kept at 4 °C until RNA isolation could be performed. RNA samples were used to determine hBD2 mRNA expression.

Saliva collection

Saliva collection was 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 (17). Saliva samples were kept at –80 °C within 2 h of collection. Samples were later thawed, mixed briefly, and analyzed for hBD2 contents using ELISA (R&D Systems, Minneapolis, MN, USA).

Tissue analysis

Reverse transcriptase polymerase chain reaction (RT-PCR)—Total RNA was extracted from tissues using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's suggestion. cDNA was prepared using 1 μg total RNA with the RETROscript kit (Ambion Inc., Austin, TX, USA). Controls without RT enzyme were included in each experiment. Amplification of the resulting cDNA was carried out with each 50 μl of PCR mixture containing 3 μl cDNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 10 mM dNTP mix, 250 nM each of forward and reverse primers, and 2.5 U of Taq DNA polymerase. Ribosomal phosphoprotein (RPO) was used as a housekeeping control gene to determine the total RNA level. PCR conditions and primer sequences for hBD2 and RPO have been previously described (18).

Quantitative real-time polymerase chain reaction (Q-PCR)—cDNA was analysed using the ABI system (Applied Biosciences, Carlsbad, CA, USA) for quantitative real-time PCR using Brilliant SYBR Green Q-PCR Master Mix (Stratagene, La Jolla, CA, USA). The reaction was set up in a 96-well plate, each well containing 12.5 μl of SYBR Green mix, 2 μl of cDNA, and 2 μM primers. The amplification conditions was initial denaturation at 95 °C for 12 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 57–65 °C for 30 s, and elongation at 72 °C for 60 s. Melt-curve analysis was performed to confirm that the detected signal was that of SYBR Green binding to the expected amplification product and not to the possible primer-dimers. Oligonucleotide primers were designed as previously described (18). In initial experiments, amplification efficiency was determined for all primer pairs. Amplification was performed in duplicate and normalized to the housekeeping gene RPO.

Quantitation of salivary hBD2 level—The levels of hBD2 protein in both unstimulated and stimulated saliva were quantified by ELISA based on matched anti-hBD2 (PeproTech,

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Rohovot, Israel). Recombinant hBD2 was used as a standard (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. Chi-square, Student's t-test, ANOVA, Wilcoxon, Kruskal-Wallis tests were employed to explore possible association between oral hBD2 and HIV/ART status according to the nature of variables. Finally, since the expression of oral hBD2 may be influenced by duration of HIV infection, level of $CD4^+$ cell count, HIV viral load, smoking and drinking behaviors, linear and logistic regression were used to further analyze factors associated with the expression of oral hBD2 mRNA and its salivary protein levels.

Significance level was set at 0.05 for all statistical tests mentioned above. All the analyses were performed using R statistical software (19).

Results

Demographic data and medical status

Tissue and saliva samples were obtained from 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). 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 and controls are shown in Table 1.

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

Prevalence of oral lesions was found to be statistically significant higher in HIV-infected subjects than non-HIV controls (Chi-square test, p<0.001) (Table 2). Significant difference was also observed with respect to ART status of the subjects. Hyperpigmentation was the most common oral lesion seen in all group of subjects. Oral candidiasis and oral hairy leukoplakia were observed in only two and one HIV-infected subjects who received ART, respectively, whereas no oral warts were observed among the subjects. Periodontal pocket depths and bleeding on probing in HIV-infected subjects were found to be statistically significant difference due to 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 different between those who were and were not on ART.

Expression of hBD2 mRNA and its salivary protein levels in HIV-infected subjects with and without ART and non-HIV individuals

Levels of hBD2 protein in both unstimulated and stimulated saliva were significantly increased in HIV-infected subjects compared to non-HIV individuals (p <0.001) (Table 2). However, no significant difference in the expression of hBD2 mRNA was observed between the two groups. Changes in the expression of hBD2 mRNA and its salivary protein levels were observed among HIV-infected subjects who received ART and those who did not. No

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significant difference was observed in the expression of hBD2 mRNA with respect to the use of ART. In contrast, levels of hBD2 protein in stimulated saliva were significant higher in HIV-infected subjects who were on ART compared to those who did not take the medication ($p < 0.001$). The levels were significantly increased in those on short-term ART compared to those who did not take the medication. The levels of hBD2 protein in stimulated saliva seemed to be decreased with long-term use of ART. However, no significant difference was observed with the levels of hBD2 protein in unstimulated saliva.

Logistic regression analysis of hBD2 mRNA expression and its salivary protein levels

On logistic regression, the levels of salivary hBD2 protein seemed to be affected by HIV infection. The levels of hBD2 protein in unstimulated saliva of HIV-infected subjects were significantly different when compared with non-HIV individuals (Table 3). No significant difference in the expression of hBD2 was observed with respect to ART status of the subjects.

Factors associated with the expression of hBD2 mRNA and its salivary protein levels

Effects of various variables on the expression of hBD2 mRNA and its salivary protein levels are shown in Table 4. The following factors were found to significantly affect the levels of hBD2 protein in stimulated saliva; duration of ART, duration of HIV infection, HIV viral load, and alcohol consumption. However, those factors were not significantly associated with the expression of hBD2 mRNA.

Discussion

This study demonstrated that oral innate immunity was affected by HIV infection and use of ART. The expression of hBD2 mRNA was increased in HIV infected subjects compared to non-HIV individuals. The levels of hBD2 protein in both unstimulated and stimulated saliva were significantly increased with HIV infection. The levels of hBD2 proteins in stimulated saliva were also found to be significantly different between HIV-infected subjects who received ART compared to those who were not on the medication.

The findings of the present study suggest that HIV infection may impair the function of oral epithelial cells in innate immunity leading to the alterations of the oral hBD2 expression. It is well accepted that HIV infection causes the impairment of both local and systemic immunity (20). Oral epithelial cells, which are part of the local innate immunity (9), may also be affected by the infection. As a consequence, various oral lesions caused by opportunistic infections and malignancies are observed among HIV infected subjects (21–23).

A recent study by Nittayananta et al (17) revealed that ART significantly decreased salivary flow rates of HIV-infected patients. Those who were on long-term use of ART had a greater risk of developing oral lesions than those with short-term ART (17). Thus, both HIV infection and long-term use of ART may have adverse effects on the systemic and local innate immunity resulting in the recurrence of opportunistic infections and increase the risk of developing oral cancers.

Although the incidence of AIDS-defining malignancies (ADMs) including Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL) has declined significantly in the ART era (24), the incidence of other malignancies not known to be associated with immunosuppression (non-ADMs) including OSCC remains significantly higher than in the general population (25, 26). Potential explanations for the higher non-ADM rates include potential oncogenicity of long-term HIV infection or of long-term ART (25). Thus, HIV-infected subjects who are on long-term ART seem to be at risk of developing OSCC (25).

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A previous study by Nittayananta et al (17) reported that both unstimulated and stimulated salivary flow rates of the subjects with ART were significantly lower than that of those without ART. Therefore, prevalence of oral *Candida* carriage is expected to be high among this group of subjects. Oral *Candida* carriage has been shown to correlate with presence of oral epithelial dysplasia (27). Chronic infection by *Candida* may cause malignant transformation resulting in the development of OSCC (28). Because HIV-infected subjects receive ART as a life-long therapy, this group of subjects may be susceptible to chronic candidal infection that could potentially lead to malignant transformation. Thus, further studies should be performed to determine the relationship between salivary hBD2 levels and the risk of developing OSCC in HIV-infected subjects on long-term ART.

Besides protective function, hBDs have evolved to be one of the most potent epithelial differentiation and tumor markers (29). Previous studies have reported altered expression of hBDs in cancers suggesting their involvement in carcinogenesis (14, 15). A study by Joly et al (14) reported that hBD1 and hBD2 mRNA expression was significantly lower in OSCC. In contrast, hBD3 was found to be overexpressed in OSCC at both transcriptional and translational levels compared to healthy oral tissue. These results suggest a putative role for hBDs in carcinogenesis and indicate that hBDs may be useful markers of OSCC. However, the specific role of hBDs in carcinogenesis is unknown.

The present study demonstrated that the levels of hBD2 in stimulated saliva were significantly different between HIV-infected subjects and non-HIV individuals. In addition, changes in the levels of hBD2 proteins in stimulated saliva were also observed with the use of ART. Interestingly, the levels of hBD2 protein in stimulated saliva seemed to be increased with short-term use of ART, but decreased with long-term use of the medication. This finding indicates that oral innate immunity may be adversely affected by HIV infection and long-term use of ART. Thus, this antimicrobial peptide may be the useful biomarker to monitor HIV-infected subjects receiving long-term ART who might be susceptible to malignant transformation of the oral mucosa.

In this study, no significant association between types of ART and the levels of salivary hBD2 proteins was observed. This may be due to the fact that most patients received the same regimen of 2 NRTIs + 1 NNRTI. AZT, a mainstay of the NRTI prescribed for HIV-infected subjects, is incorporated into DNA causing gene mutations (6). Thus, prolonged treatment by this medication potentially causes malignant transformation of oral epithelia (6). In addition, a previous study demonstrated that AZT has genotoxic effects that may lead to genomic instability in cultured cells (6). These genetic changes have been used to predict the risk of malignant transformation of oral epithelia (30). As HIV-infected subjects switch antiretroviral medications frequently, it would be interesting to determine whether long-term use of any specific medications significantly decrease the levels of salivary hBD2.

Besides HIV infection and ART, the present study demonstrated that various factors known to be associated with the development of oral cancers such as alcohol and HIV viral load, have also been shown to significantly affect the expression of hBD2. It is well accepted that alcohol consumption has strong causative links to OSCC (31). Alcohol serves as a mucosal irritant and may activate members of the cytochrome p450 family enzymes, which is known to activate procarcinogens (32). Of interest, these factors were found to be significantly associated with the expression of hBD2 only at the protein level, but not at the mRNA level. This discrepancy may indicate that the expression of hBD2 is mostly affected by those factors at the posttranscriptional level. In addition, HIV infection and ART may alter message translation or interfere with the protein synthesis.

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Infection by human papilloma virus (HPV) is also a risk factor for OSCC (33, 34). Our previous study demonstrated that the prevalence of oral HPV type 16 infection was increased in HIV-infected subjects compared to non-HIV individuals, and long-term use of ART did not seem to decrease the infection (35). Thus, further studies should be performed to determine the association between HPV type 16 infection and the expression of hBD2 that may put the subjects taking ART at risk for developing OSCC.

The strength of this study was that the expression of hBD2 was detected at both transcriptional and translational levels by using quantitative real-time PCR and ELISA, respectively. Although a causal role in tumorigenesis has not been established for hBDs, changes in the salivary levels of hBD2 may have great importance in monitoring HIV-infected subjects who are on long-term ART.

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 hBD2 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 hBD2 expression. In addition, further studies should be conducted to assess the changes in other types of hBDs produced by oral epithelial cells.

In conclusion, the present study demonstrated that oral innate immunity was affected by HIV infection and use of ART. The findings suggest that changes in oral hBD2 expression did occur in HIV-infected 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 mechanisms how HIV infection and ART alter the expression of hBD2 in oral tissue and saliva.

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References

1. Challacombe SJ, Naglik JR. The effects of HIV infection on oral mucosal immunity. Adv Dent Res. 2006; 19:29–35. [PubMed: 16672546]
2. Hammer SM, Eron JJ Jr, Reiss P, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the international AIDS society USA panel. JAMA. 2008; 300:555–70. [PubMed: 18677028]
3. Patton LL, McKaig R, Strauss R, Rogers D, Eron JJ Jr. Changing prevalence of oral manifestations of human immune-deficiency virus in the era of protease inhibitor therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000; 89:299–304. [PubMed: 10710453]
4. Nokta M. Oral manifestations associated with HIV infection. Curr HIV/AIDS Rep. 2008; 5:5–12. [PubMed: 18417029]
5. Miziara ID, Weber R. Oral lesions as predictors of highly active antiretroviral therapy failure in Brazilian HIV-infected children. J Oral Pathol Med. 2008; 37:99–106. [PubMed: 18197855]
6. Olivero OA. Mechanisms of genotoxicity of nucleoside reverse transcriptase inhibitors. Environ Mol Mutagen. 2007; 48:215–23. [PubMed: 16395695]

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7. Clifford GM, Polesel J, Rickenbach M, et al. Cancer Risk in the Swiss HIV Cohort Study: Associations with Immunodeficiency, Smoking, and Highly Active Antiretroviral Therapy. J Natl Cancer Inst. 2005; 97:425–32. [PubMed: 15770006]
8. Gillison ML. Oropharyngeal cancer: a potential consequence of concomitant HPV and HIV infection. Curr Opin Oncol. 2009; 21:439–44. [PubMed: 19587593]
9. Dale BA, Fredericks LP. Antimicrobial peptides in the oral environment: expression and function in health and disease. Curr Issues Mol Biol. 2005; 7:119–33. [PubMed: 16053246]
10. Zhao C, Wang I, Lehrer R. Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. FEBS Lett. 1996; 396:319–22. [PubMed: 8915011]
11. Mathews M, Jia H, Guthmiller J, et al. Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands. Infect Immun. 1999; 67:2740–5. [PubMed: 10338476]
12. Krisanaprakornkit S, Weinberg A, Perez C, Dale B. Expression of the peptide antibiotic human beta defensin 1 in cultured gingival epithelial cells and gingival tissue. Infect Immun. 1998; 66:4222–8. [PubMed: 9712771]
13. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003; 3:710–20. [PubMed: 12949495]
14. Joly S, Compton LM, Pujol C, Kurago ZB, Guthmiller JM. Loss of human beta-defensin 1, 2, and 3 expression in oral squamous cell carcinoma. Oral Microbiol Immunol. 2009; 24:353–60. [PubMed: 19702947]
15. Kesting MR, Loeffelbein DJ, Hasler RJ, Wolff KD, Rittig A, Schulte M, Hirsch T, Wagenpfeil S, Jacobsen F, Steinstraesser L. Expression profile of human beta-defensin 3 in oral squamous cell carcinoma. Cancer Invest. 2009; 27:575–81. [PubMed: 19219676]
16. EC-Clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Centre on Oral Manifestations of the Immunodeficiency Virus. Classification and diagnostic criteria for oral lesions in HIV infection. J Oral Pathol Med. 1993; 22:289–91. [PubMed: 8229864]
17. Nittayananta W, Talungchit S, Jaruratanasirikul S, Silpapojakul K, Chayakul P, Nilmanat A, Pruphetkaew N. Effects of long-term use of HAART on oral health status of HIV-infected subjects. J Oral Pathol Med. 2010; 39:397–406. [PubMed: 20202089]
18. Kimball JR, Nittayananta W, Klausner M, Chung WO, Dale BA. Antimicrobial barrier of an in vitro oral epithelial model. Arch Oral Biol. 2006; 51:775–83. [PubMed: 16815238]
19. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2010. URL http://www.R.project.org/
20. Challacombe SJ, Sweet SP. Oral mucosal immunity and HIV infection: current status. Oral Dis. 2002; 8(Suppl 2):55–62. [PubMed: 12164661]
21. Laskaris G, Hadjivassiliou M, Stratigos J. Oral signs and symptoms in 160 Greek HIV-infected patients. J Oral Pathol Med. 1992; 21:120–3. [PubMed: 1316436]
22. Glick M, Muzyka BC, Lurie D, Salkin LM. Oral manifestations associated with HIV-related disease as markers for immune suppression and AIDS. Oral Surg Oral Med Oral Pathol. 1994; 77:344–9. [PubMed: 8015797]
23. Nittayananta W, Chungpanich S. Oral lesions in a group of Thai people with AIDS. Oral Dis. 1997; 3(Suppl 1):1–5. [PubMed: 9456639]
24. Simard EP, Pfeiffer RM, Engels EA. Spectrum of cancer risk late after AIDS onset in the United States. Arch Intern Med. 2010; 170:1337–45. [PubMed: 20696958]
25. Bedimo R. Non-AIDS-defining malignancies among HIV-infected patients in the highly active antiretroviral therapy era. Curr HIV/AIDS Rep. 2008; 5:140–9. [PubMed: 18627663]
26. Adler DH. The impact of HAART on HPV-related cervical disease. Curr HIV Res. 2010; 8:493–7. [PubMed: 20946095]
27. McCullough M, Jaber M, Barrett AW, Bain L, Speight PM, Porter SR. Oral yeast carriage correlates with presence of oral epithelial dysplasia. Oral Oncol. 2002; 38 :391–3. [PubMed: 12076705]
28. Field EA, Field JK, Martin MV. Does Candida have a role in oral epithelial neoplasia? J Med Vet Mycol. 1989; 27:277–94. [PubMed: 2689621]

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29. Diamond G, Ryan L. Beta-defensins: what are they really doing in the oral cavity? Oral Dis. 2011; 17:628–35. [PubMed: 21332602]

30. Schaaij-Visser TB, Bremmer JF, Braakhuis BJ, et al. Evaluation of cornulin, keratin 4, keratin 13 expression and grade of dysplasia for predicting malignant progression of oral leukoplakia. Oral Oncol. 2010; 46:123–7. [PubMed: 20036603]

31. White JS, Weissfeld JL, Ragin C, et al. The influence of clinical and demographic risk factors on the establishment of head and neck squamous cell carcinoma cell lines. Oral Oncol. 2007; 43:701–12. [PubMed: 17112776]

32. Hunter KD, Parkinson EK, Harrison PR. Profiling early head and neck cancer. Nat Rev Cancer. 2005; 5:127–35. [PubMed: 15685196]

33. Herrero R, Castellsague X, Pawlita M, et al. Human papillomavirus and oral cancer: the international agency for research on cancer multicenter study. J Natl Cancer Inst. 2003; 95:1772–83. [PubMed: 14652239]

34. Mork J, Lie AK, Glattre E, Hallmans G, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med. 2001; 344:1125–31. [PubMed: 11297703]

35. Amornthatree K, Sriplung H, Mitarnun W, Nittayananta W. Impacts of long-term use of antiretroviral therapy on the prevalence of oral human papilloma virus type 16. J Oral Pathol Med. 2011 (in press).

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Table 1

Demographic data and characteristics of HIV-infected subjects with and without 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%)
Marital status				
Single	13 (23.6%)	5 (15.2%)	13 (31.7%)	21 (42.0%)
Married	33 (60.0%)	17 (51.5%)	16 (39.0%)	27 (54.0%)
Divorce	4 (7.3%)	7 (21.2%)	7 (17.1%)	0 (0.0%)
Widow	5 (9.1%)	4 (12.1%)	5 (12.2%)	2 (4.0%)
Highest education				
Primary school level	33 (56.9%)	15 (33.3%)	12 (22.2%)	24 (48.0%)
Secondary school level	18 (31.0%)	16 (35.6%)	21 (38.9%)	10 (20.0%)
Polytechnic level	2 (3.4%)	4 (8.9%)	10 (18.5%)	3 (6.0%)
University level	4 (6.9%)	9 (20.0%)	9 (16.7%)	11 (22.0%)
Other	1 (1.7%)	1 (2.2%)	2 (3.7%)	2 (4.0%)
Occupation				
Employee	31 (53.4%)	23 (51.1%)	21 (38.9%)	21 (42.0%)
Trading	6 (10.3%	5 (11.1%)	2 (3.7%)	4 (8.0%)
Agriculture	3 (5.2%)	2 (4.4%)	1 (1.9%)	8 (16.0%)
Government servant	0 (0.0%)	2 (4.4%)	9 (16.7%)	2 (4.0%)
Student	1 (1.7%)	0 (0.0%)	0 (0.0%)	8 (16.0%)
Others	14 (24.1%)	12 (26.7%)	17 (31.5%)	7 (14.0%)
Unemployed	3 (5.2%)	1 (2.2%)	4 (7.4%)	0 (0.0%)
Income (Baht)/month				
< 5,000	25 (43.1%)	18 (40.0%)	13 (24.5%)	23 (46.0%)
5,000–10,000	26 (44.8%)	17 (37.8%)	19 (35.8%)	22 (44.0%)
10,001–20,000	7 (12.1%)	6 (13.3%)	11 (20.8%)	5 (10.0%)
20,001–30,000	0 (0.0%)	2 (4.4%)	6 (11.3%)	0 (0.0%)
> 30,000	0 (0.0%)	2 (4.4%)	4 (7.5%)	0 (0.0%)
Risk group				

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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)**	
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 (year)				
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 (68.0%)
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%)
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 (2.0%)
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	-
Viral load (copies)				
Mean	782.6	21,560	5,627	-
Range	0–30,100	50–750,000	50–139,000	-

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Table 2

Oral health parameters, salivary flow rates and the expression of hBD2 mRNA and its salivary protein levels in HIV-infected subjects with and without 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							
Yes	110 (70%)	21 (42%)	< 0.001	46 (81%)	26 (57%)	38 (70%)	0.029
No	47 (30%)	29 (58%)		11 (19%)	20 (43%)	16 (30%)	
Presence of periodontal pockets							
Yes	127 (82%)	41 (85%)	0.734	51 (89%)	31 (69%)	45 (85%)	0.022
No	28 (18%)	7 (15%)		6 (11%)	14 (31%)	8 (15%)	
Presence of bleeding on probing							
Yes	146 (94%)	46 (96%)	0.736	57 (100%)	38 (83%)	51 (96%)	< 0.001
No	10 (6%)	2 (4%)		0 (0%)	8 (17%)	2 (4%)	
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	
Expression of hBD2 mRNA							
Mean (SD)	176.55 (503.68)	101.08 (239.87)	0.639	271.60 (713.91)	141.34 (371.03)	109.07 (289.37)	0.558
Salivary hBD2 levels (pg/ml)							
- Unstimulated saliva							
Mean (SD)	113.90 (101.26)	47.79 (21.30)	<0.001 *	107.35 (74.89)	116.04 (134.49)	118.03 (93.65)	0.800 **
- Stimulated saliva							

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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
Mean (SD)	269.34 (202.28)	156.32 (92.97)	<0.001 *	157.51 (109.08)	421.33 (208.96)	248.51 (187.96)	<0.001 **

* Student's t-test or Wilcoxon rank sum test if non-normally distributed

** Kruskal-Wallis test

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Table 3

Effects of HIV infection and long-term use of ART on the expression of hBD2 mRNA and its salivary protein levels based on logistic regression

hBD2	Adjusted odd ratios (95% Confidence Interval)		
	HIV-positive (HIV-negative as reference)	**Short-term ART (No-ART as reference)**	**Long-term ART (No-ART as reference)**
Expression of hBD2 mRNA in buccal mucosa >5	0.48 (0.19, 1.20)	0.77 (0.34, 1.77)	1.37 (0.61, 3.10)
Salivary levels of hBD2 protein			
- Unstimulated saliva >50	6.02 (2.69, 13.46)	0.84 (0.32, 2.19)	1.19 (0.46, 3.09)
- Stimulated saliva >100	0.87 (0.39, 1.95)	0.69 (0.29, 1.67)	1.44 (0.60, 3.47)

Table 4

Effects of long-term use of ART and other variables on the expression of hBD2 mRNA and its salivary protein levels in HIV-infected subjects

Predictors for the expression	Mean difference between groups					
	hBD2 mRNA		Salivary hBD2 protein			
	mRNA	*P*-value*	Unstimulated saliva	*P*-value*	Stimulated saliva	*P*-value*
Duration of ART						
no *vs* short-term	130.26	0.228	1.36	0.763	−263.82	0.059
long *vs* short-term	−32.27	0.635	10.52	0.733	−172.82	<0.001
Duration of HIV infection						
5–10 vs < 5 years	−150.19	0.134	8.47	0.684	−103.09	0.006
CD4 count						
<200 *vs* >= 200 cell/cm^3	−90.37	0.316	25.79	0.225	8.50	0.468
Viral load						
>= 50 *vs* < 50	−154.40	0.063	−4.69	0.914	162.11	<0.001
Smoking						
Yes *vs* No	64.80	0.160	−6.09	0.653	−22.51	0.535
Alcohol						
Yes *vs* No	−3.10	0.713	−25.8	0.397	−73.30	0.046

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

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