Review Article

Twenty Years of Oral HIV Research Experience in Thailand: Where Are We Now?

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Abstract

Acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) infection is a global health problem. Because immune cells are the target of HIV infection, the immunity of the host is compromised after being infected with the virus leading to the development of various opportunistic infections and malignancies. HIV infection affects not only systemic immunity, but also the local innate immune defense. As oral health is an integral part of general health, various oral lesions have been observed in HIV-infected individuals. Oral candidiasis is the most common opportunistic infection found in this patient group followed by hairy leukoplakia. After the introduction of highly active antiretroviral therapy (HAART), a standard treatment for HIV infection, prevalence of HIV-related oral lesions has dramatically declined. However, long-term use of HAART causes adverse effects on oral health and significantly affects oral innate immunity of HIV-infected individuals. Thus, it should be noted that even when oral lesions are not seen clinically in those on HAART, subclinical alterations of oral epithelium do occur as marked by changes in the expression of cytokeratins and innate immune mediators. In addition, decreased salivary flow rates are observed among HIV-infected individuals on HAART. Oral oncogenic virus such as Epstein-Barr virus, but not human papilloma virus, has been shown to be decreased with HAART. This review article described different aspects of research on oral health and disease in HIV/AIDS in Thailand performed by author and colleagues during the last two decades. Future directions of oral HIV research were also included.

Key words: AIDS, Candidiasis, HIV, Innate immunity, Oral lesions, Oral health, Saliva

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Introduction

Human immunodeficiency virus (HIV) infection causing acquired immunodeficiency syndrome (AIDS) has become a global health problem for more than three decades. At present, the epidemic of AIDS still continues increasing globally, particularly in Sub-Saharan Africa and Southeast-Asia including Thailand[1]. The major target cells of HIV are immune cells such as T-lymphocytes and macrophages. Therefore, after being infected by the virus, the immunity of the host is impaired leading to the development of various opportunistic infections and malignancies[2]. Because oral health is an integral part of general health, and HIV infection impairs not only the host systemic immunity but also the local innate immune defense, oral lesions in HIV-infected individuals have been frequently observed. They are classified into three groups as follows; Group I: Lesions strongly associated with HIV infection; Group II: Lesions less commonly associated with HIV infection; and Group III: Lesions seen in HIV infection[3].

Highly active antiretroviral therapy (HAART) has become a standard treatment for HIV infection and the most effective therapy of AIDS.[4] It induces a marked reduction in viral load and increase in the $CD4^+$ cell count[5] leading to a declination in morbidity and mortality of HIV-infected individuals.[6] Currently, HAART includes more than 30 different drugs of six separate classes: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, entry inhibitors, and HIV integrase inhibitors.[7]

This review article focused on different aspects of oral HIV research in Thailand performed by author and colleagues during the last two decades. Future directions for researches in this challenging field were also included.

Oral health and disease in HIV infection

Oral lesions are commonly observed among HIV-infected patients in Thailand before HAART era.[8] Pseudomembranous candidiasis was the most common lesions, followed by hairy leukoplakia.[8] Periodontal diseases in HIV infection, not as characterized by the EC-Clearinghouse,[3] were similar as those seen in general population. Of interest, non-Hodgkin's lymphoma was the only detected neoplasm,[8] and may manifest as delayed healing of extracted wound.[9] Kaposi's sarcoma, the most common neoplasm seen in AIDS patients in western countries, was not noted in a group of Thai people with AIDS.[8] Deep fungal infections including histoplasmosis and penicilliosis were detected.[8,10] Xerostomia was found to be a common condition in this patient group.[8]

Following the introduction of HAART, prevalence and severity of opportunistic diseases in HIV-infected patients are dramatically decreased.[4] Several studies reported that the frequency and characteristic of HIV-related oral manifestations have changed as a result of HAART.[11-13] In Thailand, prevalence of oral lesions has also been dropped significantly after the wide use of HAART among HIV-infected individuals.[14] Oral candidiasis and hairy leukoplakia were found in 54 % and 13 % before the introduction of HAART versus 2 % and 1 %, respectively following HAART.[8,14]

Of interest, while studies in western countries reported an increased prevalence of oral warts caused by human papilloma virus (HPV) among those on HAART,[11,15-18] the lesions were not observed in HIV-infected individuals in Thailand.[14] In addition, the lesions have never been reported from studies in other Asian countries.[19,20] It is not known why oral warts are uncommon in Asian population. Further studies should be performed to determine whether there are any differences between those two groups of population in terms of genetics and host susceptibility to the virus.

Due to the wide use of HAART among HIV-infected individuals, its adverse effects have been explored.[14] A study by Nittayananta *et al*[14] reported that oral health of HIV-infected individuals is improved with short-term use of HAART, but long-term use of HAART seemed to have some adverse effects. HIV-infected patients

without HAART and those on long-term use of HAART seemed to have a greater risk of developing cervical caries than those with short-term use of the medication.[14] According to periodontal health, those not on HAART had a greater risk of having periodontal pockets of depth ≥ 4 mm than those with HAART.[14] In western countries, prevalence of HIV-associated periodontal disease has also been reported to decrease significantly with HAART.[11,12,21,22] A study by Baqui *et al*[23] reported that greater levels of periodontal destruction were associated with higher HIV viral loads. However, a biological explanation for this association remains unclear. Further studies are needed to clarify this relationship.

Regarding oral symptoms, HIV-infected individuals not on HAART showed greater risks of having orofacial pain, and oral lesions than those with HAART.[14] A previous study by Patton *et al*[24] also reported that oral symptoms were frequently observed among HIV-infected individuals and may have a significant impact on their quality of life.[25,26] Thus, oral health care professional plays a crucial role in improving and maintaining health-related quality of life in HIV-infected individuals.

Saliva in HIV infection

Saliva plays a key role in maintaining oral health and protecting oral tissues.[27,28] It is well established that salivary glands are affected during the course of HIV infection.[29,30] Complaints of dry mouth or xerostomia have been reported as a common condition among the infected individuals, varying from 7 % to 63 % depending on study population and geographic locations.[8,31,32] Systemic diseases and xerogenic medications are common causes of salivary gland hypofunction.[33,34] However, subjective complaints of dry mouth may not imply objectively measurable diminished gland function.

Hyposalivation, xerostomia, and oral health status were assessed in a group of HIV-infected Thai people before HAART era.[35] The unstimulated flow rates in HIV-infected patients were significantly lower than non-HIV controls. However, no significant difference between the groups was found with respect to stimulated flow rate. Of interest, hyposalivation was significantly associated with the colony forming unit of *Candida*. Smoking and alcohol consumption were significantly associated with hyposalivation, but not xerostomia. Other factors including sex, stage of HIV infection, risk group of HIV infection, systemic disease, and medication use have been reported to be significantly associated with both hyposalivation and xerostomia.[35]

A correlation between reduced whole saliva secretion rate and subjective feelings of oral dryness has been reported.[36,37] Subjective feeling of oral dryness might be a result of salivary gland hypofunction.[38] However, absence of complaint of dry mouth does not indicate adequate salivary gland function and vice-versa.[35] Without additional salivary gland evaluation, it may be difficult to determine if a given individual has salivary gland hypofunction. Therefore, the associating saliva flow rate with subjective complaints of oral dryness and responses of subjects to different questions may help to identify the patient with hyposalivation.[39,40]

Relationship between self-reported on xerostomia and salivary flow rates among HIV-infected individuals was assessed.[41] Complaint of dry mouth or xerostomia was shown to be common among HIV-infected individuals.[41] Responses to the questions “Do you carry water or a saliva substitute ?” and “Have you had taste disturbance ?” were significantly different between HIV-infected individuals and non-HIV controls. Subjects’ responses to questions concerning dry mouth were significantly correlated with low unstimulated salivary flow rate. A significant correlation between visual analogue scale (VAS) of feeling of dry mouth and salivary flow rates was also noted.[41] Positive responses to self-reported xerostomia questions reflected low unstimulated salivary flow rate.[42] Thus, it is suggested that questions concerning dry mouth may be useful tools to identify HIV-infected individuals with hyposalivation, especially at a resting stage.[41]

In HAART era, a study by Nittayananta *et al*[14] reported that salivary flow rates of both unstimulated and stimulated saliva in HIV-infected individuals were statistically

significant lower than non-HIV controls. The flow rates of those on HAART were also significantly lower than those not on the medication.[14] A study by Lin *et al*[42] reported no changes in the salivary flow rates of HIV positive men on HAART compared with those not on HAART, whereas a study by Navazesh *et al*[43] revealed that PI based HAART was significantly associated with a decrease in both unstimulated and stimulated salivary flow rates. Xerostomia and lipodystrophic changes of the salivary glands have previously reported as potential adverse effects of PI therapy.[44] It has been proposed that the chemical structure of the PI may alter the structure and composition of saliva thereby decreasing the salivary flow of HIV-infected individuals receiving HAART.[45]

A study by Nittayananta *et al*[14] reported that CD4 cell count and HIV viral load were not statistically significant associated with salivary flow rates. In contrast, a study by Navazesh *et al*[43] indicated that the reduction of CD4 cell counts and the increased HIV viral load were significantly associated with reduced salivary flow rates of HIV-infected individuals. Lymphoproliferative response, as a result of high levels of HIV p24 antigen, may be an important risk factor for reduced salivary flow rates among those subjects.[46]

Regarding xerostomia, HIV-infected individuals not on HAART showed a greater risk of feeling dry mouth than those with short-term HAART.[14] A previous study by Silverberg *et al*[47] reported that prevalence of xerostomia was increased in patients who had discontinued HAART and those who had switched its regimens. Those patients with stable HAART as well as those who continued using HAART for at least 6 months had low prevalence of xerostomia.[43,47] However, long-term HAART seemed to have adverse effects on salivary flow rates.[14] Since the exact nature of the changes in salivary gland structure and function with HAART remains unknown, further studies should be performed to determine the effects of long-term use of HAART on salivary gland structure and function.

Challenges in treatment of oral candidiasis in HIV infection

Oral candidiasis caused by *Candida* species is a common opportunistic infection observed in HIV-infected individuals both before and following the introduction of HAART.[8,14] Colony forming unit (CFU) of oral *Candida* has been shown to be a good indicator of immune defects among individuals at high risk for developing AIDS.[48] The CFU of oral *Candida* was found to be higher among AIDS than that of the asymptomatic or symptomatic HIV-infected individuals.[49]

Although various topical and systemic antifungal drugs are available,[50] treatment of oral candidiasis in HIV-infected individuals remains a challenge. Due to underlying immune deficiency of the host, high recurrence rate is frequently noted after cessation of the medication.[50] Thus, control of the symptoms rather than cure may be the goal in the treatment of oral candidiasis in HIV infection. In addition, it is important to find interventions that can prolong the time to relapse of the lesions.

It is well accepted that adhesion of *Candida* to the mucosal surfaces is a vital step for successful colonization and infection. Chlorhexidine (CHX) is capable of inhibiting candidal adhesion to the mucosal surfaces,[51] and has been shown to possess antifungal activity both *in vitro* and *in vivo*.[52,53] In addition, it seemed to be useful in treating as well as preventing oral candidiasis in HIV-infected children.[54] A study by Nittayananta *et al*[55] reported that 0.12 % CHX may be a useful mouthwash in the maintenance of OC-free period in HIV infection. However, bitter taste and tooth staining of CHX may cause poor compliance. Moreover, desquamative lesions and soreness of the oral mucosa have also been reported.[56] Thus, mouthwash containing herbal plant with antifungal activity may be developed and determined whether it can be used as an alternative mouthwash for prophylaxis of oral candidiasis in HIV-infected individuals.

Lawsone methyl ether (2-methoxy-1,4-naphthoquinone) (LME) isolated from *Impatiens balsamina* L.[57] and *Swertia calycina*[58] has been shown to possess

potent antifungal and antibacterial activities.[59,60] A previous study *in vitro* reported that 0.5 % LME preparation in oral base has antifungal activity similar to 1 % clotrimazole cream.[61] No skin rash in rats was observed up to 5 days after application.[61] Antifungal activity of 0.025 % LME mouthwash was evidenced up to 2 hours although significantly lower than that of 0.12 % CHX.[62] Patients using LME mouthwash showed no allergic reaction, and graded to have less bitter taste and greater satisfaction on its taste and smell than CHX mouthwash. In addition, a recent study by Nittayananta *et al*[63] revealed that neither antifungal drug resistance nor significant changes in genotyping of *Candida* were noted among those receiving LME mouthwash for two weeks. As ideal antifungal drugs for treating oral candidiasis is not yet available and LME has been shown to possess potent antifungal activity against oral *Candida* in HIV-infected individuals,[63] its formulation as mouthwash may be used as an intervention after successful treatment of oral candidiasis in HIV infection.[62]

Oral epithelial cells in HIV infection and HAART

Epithelial cells lining oral mucosal surfaces form important barriers against invasion of microorganisms. The cells comprise of cytokeratins (CKs), which are intermediate filament cytoskeletal proteins that provide their integrity.[64] CKs are divided into two subfamilies that are co-expressed during epithelial cell differentiation. In normal stratified squamous epithelia as found in buccal mucosa, the entire suprabasal compartment is comprised of CK4 and CK13, whereas the basal compartment expresses CK5 and CK14.[64]

Although oral lesions are dramatically decreased in those receiving HAART, oral epithelial tissue may be sub-clinically changed. A study by Nittayananta *et al* [65] demonstrated that oral epithelial tissue is altered microscopically both in HIV infection and HAART. The expression of oral epithelial biomarkers such as CK13 and CK14 was found to be altered by HIV infection and long-term HAART.[65] In HIV-infected individuals, CK13 and CK14 seemed to be less expressed with a weak staining compared with non-HIV controls.[65] In addition, their expression was affected by HAART[65] suggesting that although HIV-related oral manifestations are dramatically decreased among those on HAART, subclinical alterations of oral epithelial tissue do occur.[65] As CKs are important for the mechanical stability and integrity of epithelial cells and tissues, changes in the expression of CK13 and CK14 may imply that HIV infection and HAART impair the cell integrity and function as a protective barrier. This may ultimately lead to development of various opportunistic infections as adverse effects of long-term HAART.

Oral innate immunity in HIV infection

Oral epithelial cells are parts of mucosal innate immunity. The cells provide not only a physical barrier but also produce different antimicrobial peptides including human β-defensins (hBDs), secretory leukocyte protease inhibitor (SLPI) and various cytokines that play a crucial role in the regulation of oral infection and cancer.[66] Both hBDs and SLPI exhibit broad antimicrobial activities and help in maintaining oral homeostasis.

Oral innate immunity is affected by HIV infection and long-term use of HAART.[67,68] The expression of hBD2 and SLPI was significantly changed in HIV infected individuals compared to non-HIV controls.[67,68] The levels of their proteins in saliva were also found to be significantly different between those on HAART compared to those not on HAART. In addition, salivary pro-inflammatory cytokines, which are parts of oral innate immunity, have been shown to be affected by HIV infection and HAART.[69] Differences in salivary cytokine profiles including TNF-α and IL-8 were also observed suggesting that HIV infection and HAART may have adverse effects on the local innate immunity.[69] As a consequence, various opportunistic infections and malignancies are observed among HIV-infected individuals even in the HAART era.[11,70,71]

Oral oncogenic viruses in HIV infection

An increased risk for malignancy of different

organs including oropharynx has been reported among HIV-infected individuals on HAART.[70,71] This may be due to an increased prevalence of oral oncogenic virus infection such as HPVs after the initiation of HAART.[72] In addition, it has been shown that HAART increased the prevalence of HPV-associated oral cancers.[73] Because HIV-infected individuals received HAART as a life-long therapy, long-term use of HAART may have adverse effects on the host oral innate immunity against HPVs and thus increase the prevalence of the viral co-infection.

In general, HPVs are classified into two groups; low risk and high risk types.[74,75] HPV-16, classified as high-risk species, is usually involved in epithelial carcinogenesis. It is found in premalignant and malignant lesion and is identified in 90 % of HPVs associated head and neck squamous cell carcinoma (HNSCC) and in 50 % of oropharyngeal SCC.[76]

The prevalence of HPVs infection and HPVs-associated diseases including oral SCC are greater in HIV-infected individuals when compared to non-HIV controls.[77,78] Patients with HPV-16 positive surveillance salivary rinses are at high risk for development of recurrence and distant metastasis of HNSCC indicating that an immunologic impairment may contribute to cancer development.[79]

Malignant transformation caused by HPV-16 is mediated through the expression of *E6* and *E7*. By using quantitative polymerase chain reaction (Q-PCR) assay to detect HPV-16 *E6* and *E7* in saliva, prevalence of oral HPV-16 infection was found to be significantly higher in HIV-infected individuals than non-HIV-controls.[80] However, HAART and its duration did not significantly affect the prevalence and the copy numbers of the virus.[80]

Epstein-Barr virus (EBV), a double-stranded DNA virus in the Gamma herpesvirinae subfamily,[81] is another oncogenic virus that can cause malignancy including NHL in HIV-infected patients.[82,83] It has been shown that individuals who are infected with HIV have greater risk for developing NHL compared with that in the general population.[70] A disseminated oral EBV infection in HIV-infected individuals after the initiation of HAART has been reported[84] and that oral EBV infection may be related to the differentiation of tissue to cancer.[83] By using Q-PCR assay targeting disparate but highly conserved segments of the EBV genomes including *BamH1W*, it has been shown that HIV-infected individuals had significantly higher levels of EBV in saliva than non-HIV controls.[85] HAART significantly decreased the levels of oral EBV among HIV-infected individuals.[85] Those who were on long-term HAART had significantly lower levels of oral EBV than those on short-term HAART.[85] However, no significant difference between the groups was observed when using *EBNA1* assay.[85]

Due to its pathogenicity, EBV is likely to be co-infected with HIV[86] and is frequently shed asymptomatically in saliva of HIV-infected individuals.[87,88] It should be noted that oral EBV was also observed in the general population.[47,89] However, high prevalence of EBV in saliva of HIV-infected individuals may be due to the suppressed immune system that reduced the ability to control the viral replication. The co-infection of EBV and HIV may also be due to a lack of immunosurveillance by virus-specific $CD8^+$ cytotoxic T lymphocytes and virus specific $CD4^+$ T cells.[90,91] Because of loss of immune control on viral replication and transformation, HIV infection may amplify the effects of EBV to promote development of cancer including NHL.[92] As strong correlation between the oral EBV load and that in the peripheral blood cells has been reported,[93] levels of EBV in saliva may be a useful marker to early detect HIV-infected individuals on long-term HAART who are at risk for developing oral NHL.

Conclusion

In conclusion, HIV impairs both systemic and local immunity of the host. Thus, oral lesions are common among HIV-infected individuals. Before the introduction of HAART, oral candidiasis is the most common oral lesion followed by hairy leukoplakia. Prevalence of oral lesions is dramatically decreased in HAART era. Although oral lesions are not seen clinically in those on HAART, subclinical alterations of oral epithelium do occur as marked by changes in the expression of CK13 and CK14. Long-term use of HAART seems to have adverse effects on oral health, and significantly affect oral innate immunity of HIV-infected

individuals as shown by the alterations in the expression of hBD2, SLPI and some cytokines. The immune deficiency caused by HIV also increased the prevalence of oral oncogenic viruses including EBV and HPV-16. HAART tends to decrease the levels of EBV but not HPV-16. This may be due to direct impacts of HAART on the reactivation and replication of EBV reflecting in their viral load. It may also be explained by the partial reconstitution of the immune system that HAART-treated individuals might have an incomplete control over the reactivation of HPV-16.

Future directions

Based on the author's experience, future directions of oral HIV research should include the following issues. Due to the underlying immune deficiency of the host, treatment of oral candidiasis is still challenging and remains a major oral health problem in HIV-infected individuals. Those patients tend to have oral candidiasis as a chronic opportunistic infection even in HAART era. Thus, more research on herbal plants with antifungal activity should be performed to reduce the use of antifungal drugs that may lead to the risk of drug resistance. In addition, further studies should focus on some herbal plants that could enhance oral innate immunity of the host as another strategy to fight against candidal infection. Because HIV-infected individuals have to be on HAART as a life-long therapy and recent studies reported that HAART may cause malignancies of different organs, longitudinal studies should be performed to determine if long-term use of HAART lead to the development of oral cancer. Furthermore, as prevalence of oral HPV-16 infection has been shown to be significantly higher in HIV-infected individuals than non-HIV-controls, preventive strategies of oral HPV-16 infection should be developed to reduce the risk of oral cancer in those patients.

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