Materials and methods

A cross-sectional study was performed in the two different regions of Thailand; in the north at three provincial hospitals in Chiang Rai, Lampang, and Phayao provinces during a 10-day period in April 1997, and in the south at the Dental Hospital, and Medical Hospital of the Prince of Songkla University, and Hat Yai Regional Hospital in Songkhla province during May 1997-August 1997. The study population comprised 80 HIV-seropositive individuals who were at least 18 years of age and 58 HIV-seronegative individuals served as controls. Patients with local radiation therapy on

head and neck region were not included. The following data of each patient were recorded on a special form: age, gender, risk group of HIV infection, stage of HIV infection, immune status, systemic diseases and pharmaceutical consumption, feeling of dry mouth.

Ethics

The study protocol was approved by the research committee of the Prince of Songkla University, 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 and written consent obtained. However, they were allowed to leave the study at any time during the procedure. Persons were excluded if they were severe ill, incompetent or if the informed consent was not obtained. HIV blood testing was not done for the non-IVDU subjects due to ethical reasons.

Subjects:

HIV-seropositive individuals

Eighty individuals who were previously diagnosed as 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 1/2 Plus, Behring, Behringwerke AG, Marburg, Germany), were asked to participate as cases. Seventeen persons refused to participate in the study and 7 of them decided to leave during the study procedure, resulting in 56 HIV-seropositive subjects. They were categorized into three stages of HIV infection according to WHO criteria (WHO 1992); asymptomatic (n=13), symptomatic (n=17),

and AIDS (n=26). Two groups of persons were identified according to their risks of becoming HIV infected: 1) non-intravenous drug users (non-IVDU; n= 43) including heterosexual (n= 41), homosexual (n=1), and blood transfusion (n= 1), and 2) intravenous drug users (IVDU; n=13). The subjects belonged to non-IVDU group were those who had been hospitalised in a medical ward or came for medical care at an outpatient clinic of those included hospitals in northern and southern Thailand. The IVDU group consisted of individuals who came to receive methadone at an IVDU Unit of Hat Yai Regional Hospital in Songkhla province in the south of the country.

HIV-seronegative individuals

Thirty non-IVDU persons who had attended the Dental Hospital of the Prince of Songkla University Hospital for receiving routine dental treatment, and 33 volunteers of the sixth year dental students of the Prince of Songkla University were asked to participate as controls. As all IVDU who attended the IVDU Unit of Hat Yai Regional Hospital had to be tested routinely for HIV seropositivity, 21 persons who were proved to be HIV-seronegative were asked to participate as controls. Five of them refused to participate in the study, resulting in 79 HIV-seronegative controls. They were grouped to be non-IVDU (n=63), and IVDU (n=16), and subdivided into groups of persons with (a) or without known systemic diseases (b).

Various characteristics of HIV-seropositive and seronegative individuals are shown in table 1.

Determination of immune status

Immune status of all subjects was determined by the total lymphocytes per mm^3 as an alternative marker because facilities for determination of $CD4^+$ T-cell counts were not available (WHO 1992). In some cases the total number of lymphocytes per mm^3 was already determined within a 3-month period, in such cases these values were used. Grouping of HIV-seropositive and seronegative individuals according to total lymphocyte cell counts (cell/mm^3) is shown in table 2.

Medications

Pharmaceutical consumption was assessed by interview and/or looking through the patients' medical record. The medications taken by individuals at the day of examination and saliva collection were grouped as 1) xerostomia-inducing drugs by product category according to Sreebny and Schwartz (Sreebny and Schwartz 1986), and 2) drug group which has not been known of xerostomic side effect i.e. antibacterial, antifungal, antiviral drugs etc. All IVDU had received methadone daily at the time of examination and saliva collection. Table 3 shows groups of medications used among the patients.

Measurement of saliva flow rate

A measurement of saliva flow rate was conducted only in the morning between 9:00 a.m.-12:00 a.m.. All patients were asked to rinse their mouth with 0.9% normal saline and split out, and thereafter swallow before starting the collection procedure. The measurement comprised unstimulated and wax-stimulated whole saliva flow rate

using the draining technique as described in detail by Navazesh 1982 (Navazesh and Christensen 1982). In brief:

Unstimulated whole saliva was collected over 15 minutes. The subjects were instructed to lean their body forward and drain their saliva passively into a pre-weighed plastic cup by using a digital weight (OHAUSE, USA) for a period of 5 minutes. They were asked to repeat the procedure for two times more by using another two pre-weighed plastic cups and collecting saliva for 5 minutes. One gram of saliva is assumed to be equal to 1 mililiter of saliva. The unstimulated saliva flow rate (ml/min) was calculated by using a mean weight of the three collected saliva samples and dividing it with 5 minutes.

Stimulated saliva was collected over 6 minutes. The subjects were instructed to chew a cubic of 1 gram paraffin without taste with a frequency of 25 times per minute for 2 minutes. The subjects were instructed not to swallow saliva during the course of saliva collection. Persons with denture were asked to chew a cubic of paraffin was without removal of their denture. The patients passively drained their saliva into a pre-weighed plastic cup four times at every 30 seconds. They were asked to repeat the procedure for two times more by using another two pre-weighed plastic cups and collecting saliva for 2 minutes. The stimulated saliva flow rate (ml/min) was calculated by using a mean weight of the three collected saliva samples and dividing it with 2 minutes.

Oral candidiasis

Clinical diagnosis of oral candidiasis will be made according to the criteria classified by the EC-Clearinghouse in 1993 (EC-Clearinghouse 1993).

Microbiological assessment

Oral rinse specimens using 10 ml sterile phosphate buffered saline (PBS) were obtained from each subject according to the method of Samaranayake *et al.* (Samaranayake *et al*, 1986). The oral rinse was centrifuged at 3,000 rpm 5 min of sterile PBS. Suitable dilutions were inoculated on Sabouraud's dextrose agar (Samaranayake and Holmstrup 1989). And inoculated at 37°C for 48 h. Yeast-like colonies were counted and 1-20 isolated from each plate identified as the basis of chlamydospore and germ tube production, carbohydrate formation and assimilation.