CHAPTER III

PROPOSAL

9 month and 3 years Incidence of Tuberculosis and Associated Factors Among HIV-infected Persons Registered for 9-month Isoniazid Preventive Therapy in Chiang Rai, Thailand

3.1 Introduction

3.1.1. HIV/TB burden

HIV infection is a potent risk factor for reactivation of *Mycobacterium tuberculosis* infection. The association between TB and HIV presents an immediate and grave public health and socioeconomic threat, particularly in the developing world. About a third of the world's population (around 2 billion) carries the TB bacteria and about 11 million people are dually infected with TB and HIV in worldwide. In South East Asia, TB co-infection rate of HIV infected persons is between 56 and 80 %.

In Thailand, the prevalence of active TB decreased until 1991, but after that active TB

tended to rise slightly and is becoming a significant health problem as a result of the HIV/AIDS epidemic. Each year, an estimated 15,000 new active TB cases occur and active TB becomes the most frequent opportunistic infection among HIV infected persons. (Ministry of Public Health, 2000)

Chiang Rai province is in the northernmost area of Thailand and one of the highest HIV epidemic areas. HIV prevalence in active TB patients and antenatal clinic (ANC) attendees in Chiang Rai Hospital is shown in Table 3.1. HIV prevalence in active TB patients was 40 to 50 persent after 1993. HIV prevalence in ANC attendees was maximum 7.9 % in 1994 but recently decreasing trend. (Siriarrayapon P., 2000)

Table 3.1. HIV prevalence in active TB patients and ANC in Chiang Rai Hospital from 1990 to 1999.

Year	HIV prevalence among active TB patients (%)	HIV prevalence among ANC attendees (%)
1990	3.89	3.8
1991	11.31	5.8
1992	22.75	6.5
1993	41.94	6.4
1994	40.77	7.9
1995	47.37	6
1996	51.88	7.2
1997	52.23	7
1998	50.89	6
1999	42.06	4.23

3.1.2. Active TB risk among HIV infected persons

Infection with HIV has changed the natural history of infection with *Mycobacterium tuberculosis*. Usually no more than 10 % of people infected with TB develop active TB during lifetime. But when healthy carriers of the TB bacteria get HIV infection, their lifetime risk of developing active TB increased to over 30 times. (UNAIDS, 2000).

Overall annual risk of developing active TB in persons infected with only tubercle bacillus is 0.4 %, but in persons co-infected with TB and HIV, annual risk of developing active TB rise to 8% (WHO and UNAIDS, 1998). Thus, preventive treatment may be an important intervention to reduce the burden of active TB in HIV infected persons.

3.1.3. Isoniazid preventive therapy (IPT)

Isoniazid preventive therapy is the use of Isoniazid antituberculosis drug given to individuals with infection with *Mycobacterium tuberculosis* in order to prevent the progression to active TB. HIV is the most powerful known risk factor for progression from infection with *M. tuberculiosis* to active TB. So, to prevent the development of active TB among TB/HIV co-infected persons is important. (WHO, 1999)

IPT for tuberculin test positive HIV infected persons living in areas with high TB prevalence will reduce the risk of developing active TB in short term to around 40 % of what it would have been without such treatment (WHO/UNAIDS, 1999). In tuberculin skin test negative HIV infected persons, the efficacy of IPT remains unproven. WHO has recommended that people found to be seropositive for HIV should be screened for TB infection with tuberculin skin test and that preventive therapy should be considered for those with a positive result.

3.1.4 Situation of IPT in upper northern Thailand.

For the communicable disease control (CDC), Thailand is divided into 12 regions. The upper northern part called CDC region 10, comprises of 6 provinces: Chiang Rai, Chiang Mai, Lampang, Lamphun, Payao and Mae Hongson and there are 71 hospitals under the Provincial Health Office of the MOPH. This region showed the highest HIV-seropositive rate in new active TB patients in the country. A mail and phone call survey about information of IPT was conducted in 71 government hospitals in region 10 in 1999. 68 (96 %) out of 71 hospitals responded. The result was that, 33 hospitals (46%) providing IPT and a total of 4121 HIV infected persons were enrolled for IPT during 1994 to 1998. Data on IPT outcome since the initiation of IPT until 1999 were

available from 2370 HIV infected persons of 24 hospitals as following; 1100 (46 %) HIV infected persons completed treatment, 807 (34%) interrupted treatment for more than 2 consecutive months (default) and 414 (18 %) had died.

3.2. Rationale of the study

For rationale of this proposal, four problems and consequences are described below.

3.2.1. Improper screening is related to development of active TB among HIV infected persons registered for IPT.

CDC (1995) recommended that all people being considered for preventive therapy should receive a medical evaluation as a pre-IPT screening. One reason for this pre-IPT screening is to exclude the possibility of active TB, because active TB cases should be given treatment for active TB, not IPT for TB infection. Another reason for pre-IPT screening is to exclude tuberculin skin test negative cases and symptomatic HIV infection. Because meta-analysis suggested that the effect of IPT was restricted to tuberculin skin test positive persons (Bucher HC et al.,1999). Also, Mwinga A et al. (1998) reported IPT might be more effective in HIV infected persons with less advanced immunosuppression.

To rule out the possibility of active TB and symptomatic HIV infection, clinicians should evaluate the potential IPT participants with a proper pre-IPT screening method like tuberculin skin test, sputum smear, culture and chest x-ray. Although, study in northern Thailand shows that some IPT participants did not be evaluated with a proper pre-IPT screening method. Also, symptomatic HIV infected persons and active TB cases were included to IPT. These cases have a higher probability to develop active TB. (Communicable disease control region10, 2000)

3.2.2. Long-term IPT efficacy is not clear

Study of 131 HIV infected persons who received over 9 month-IPT in Spain, 8 participants developed active TB during a median follow-up of 43 months. They conclude that IPT provides a long term benefit in HIV infected persons, (0.61 per 100 patient-years), with a cumulative probability of active TB is less than 5 %, 3 years after completed 9-month IPT. (Casado J L et.al.,2002) This low rate of active TB after IPT is similar to that described in a small study of 29 HIV infected persons who were completing 9 – to 12 – month IPT and followed for a median of 89 months. (1.6 per100 patient-year) (Moreno S. et al., 1997)

But the study in northern Thailand; Akarasewi P, et al, (1999) reported that from 324 HIV infected persons who completed 9-month IPT, 2 cases of active TB occurred at 9-12 months after IPT and 5 cases at 18-24 months after IPT. They concluded that, IPT has very good protective effects at the first 18 months. However, the risk of active TB at 2 years or later seems to be high.

Another report in Zambia shows that, the efficacy of IPT falls with increasing time, and after 18 months, there is less of an effect. The incidence rate of active TB among HIV infected persons with IPT is a half of HIV infected persons without IPT during 6 months since IPT enrollment. But the difference of incidence rate of active TB between IPT and non- IPT groups decreased after 18 months since IPT enrollment. (Table 3.2) These results are based on a small number of events and the test for interaction was not statistically significant.

Table 3.2. Incidence rate of active TB in the time since IPT enrollment

Time since IPT enrollment to develop active TB (Months)	**No IPT** (Rate per 100 person years of follow-up)	**6-month IPT** (Rate per 100 person years of follow-up)
0 - 5.9	2.82	1.46
6 – 17.9	4.95	0.90
Over 18	6.59	5.85

(A. Mwinga et al. 1998.)

So, long-term IPT benefit after over 18 months since IPT enrollment is not clear.

3.2.3. Incidence of active TB depends on diagnostic method

A. Mwinga et al. (1998) reported that the incidence rate of confirmed and presumed active TB among 6-month IPT was 2.74 per 100 person year, but after including probable active TB, the incidence rate increased 4.94. The classification of active TB diagnosis used in this study is described below.

1) Confirmed active TB; Smear or culture or histopathology prove active TB.

2) Presumed active TB; Pulmonary infiltrates and clinical symptoms or pleural or pericardial effusion without a response to antibiotic but with a response to active TB treatment within 2 months.

3) Probable active TB; Radiological features and respiratory symptoms suggestive of active TB, who were started active TB treatment before antibiotics were given.

3.2.4 There is a lack of understanding of the factors related to development of active TB in Thailand.

There are some reports about factors related to development of active TB among HIV infected persons prescribed IPT world wide, but there is no research in Thailand. Some factors that have reported to decrease or increase active TB incidence are described

below. The evidence to select these factors has written in essay part too. (P20)

1. Factors which decrease active TB incidence

- Lymphocyte counts of 2 X10 ^{9}/l or higher

- Hemoglobin of 10 g/dl or higher

2. Factors which increase active TB incidence

- Age more than 30 years old.

- Exposure to active TB

- No BCG vaccine scar

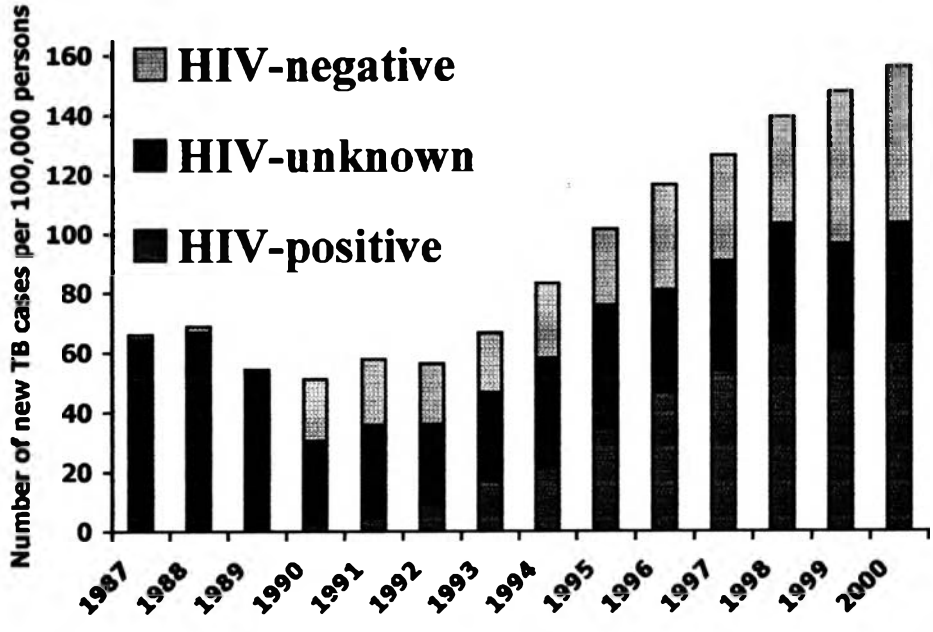
- CD 4 lymphocytes count less than 20

(A. Mwinga et al. 1998, Casado J L et.al. 2002)

3.3 Background of the study site

Chiang Rai is the northernmost province of Thailand, bordering Myanmar and Laos in an area as a Golden Triangle. Chiang Rai has a population of 1.2 millions. New active TB rate in Chiang Rai province by the Chiang Rai provincial health center were shown in Figure 3.1. From this figure, new active TB cases were rapidly increased from 1992, due to the serious impact of HIV epidemic.

Figure 3.1. New active TB rate (pre 100,000 persons) and HIV seroprevalence among new active TB cases in Chiang Rai province in 1987 - 2000



3.4 Research questions

1. What is the 9-month incidence rate of active TB among HIV infected persons who registered for 9-month IPT in Chiang Rai province?

2. What are the factors affecting the development of active TB among HIV infected persons who registered for 9-month IPT in Chiang Rai province?

3. What is the 3-year incidence rate of active TB among HIV infected persons who

completed 9-months IPT in Chiang Rai province?

4. What are the factors affecting the development of active TB among HIV infected persons who completed 9-month IPT in Chiang Rai province?

Definition of 9-month IPT completion in this study is that; when 9-month IPT finish, participants are followed-up by each hospital without default. The number of Isoniazid pills, which they have taken during 9 months, is not related to the condition of 9-months IPT completion.

3.5 Objectives

3.5.1. General Objectives

To influence to the long-term development of preventive measures of active TB among HIV infected persons in Chiang Rai province, Thailand.

3.5.2. Specific Objectives

1. To determine 9-month incidence rate of active TB among HIV infected persons

registered for 9-month IPT in Chiang Rai province.

2. To identify the factors affecting the development of active TB during 9-month IPT among HIV infected persons who registered for 9-month IPT in Chiang Rai province.

3. To determine 3-year incidence rate of active TB among HIV infected persons who completed 9-months IPT in Chiang Rai province.

4. To identify the factors affecting the development of active TB after 9-month IPT among HIV infected persons who completed 9 months IPT.

3.6. Implications of the study

1. To develop the optimal IPT guideline of active TB diagnostic method for HIV infected persons.

2. Health care workers are encouraged to pay more attention for IPT participants who have factors related to development of active TB

3.7. Methods of study

3.7.1. Study design

This study will be a prospective cohort study

3.7.2. Study duration

Enrollment: From July 2002 until the sample size is reached. (Expected 3 months)

Follow-up: 9-month IPT plus 3 year follow up after 9 months, for a total of 45 months.

3.7.3. Study population

1. For study of 9-month incidence:

   HIV infected persons registered for 9-month IPT in all public hospitals in Chiang Rai province.

2. For study of 3-year incidence:

   HIV infected persons who completed 9-month IPT in all public hospitals in Chiang Rai province.

There 16 district hospitals and 1 provincial hospital in Chiang Rai province.

(Mae Chan, Mae Sai, Chiang Sean, Wiang Chai, Phaya meng rai, Khun Tan, Mae Lao, Phan , Padad, Mae Pha Luang, Wiang Chiang Rung, Wiang Papao, Chaing Khong, Theong, Wiang Kan, Mae Sruay, Chiang Rai hospital.)

The number of newly diagnosed HIV infected persons is about 100 per every month. Also, the cumulative number of HIV infected persons who have no history of IPT from 1995 is 2660. This research recruits participants from both newly and previously diagnosed HIV infected persons without history of IPT.

3.7.4. Inclusion criteria

- Asymptomatic HIV infected persons registered for IPT in Chiang Rai.
- Tuberculin skin test positive HIV infected persons.
- HIV infected persons who are willing to participate in this study.

3.7.5. Exclusion criteria.

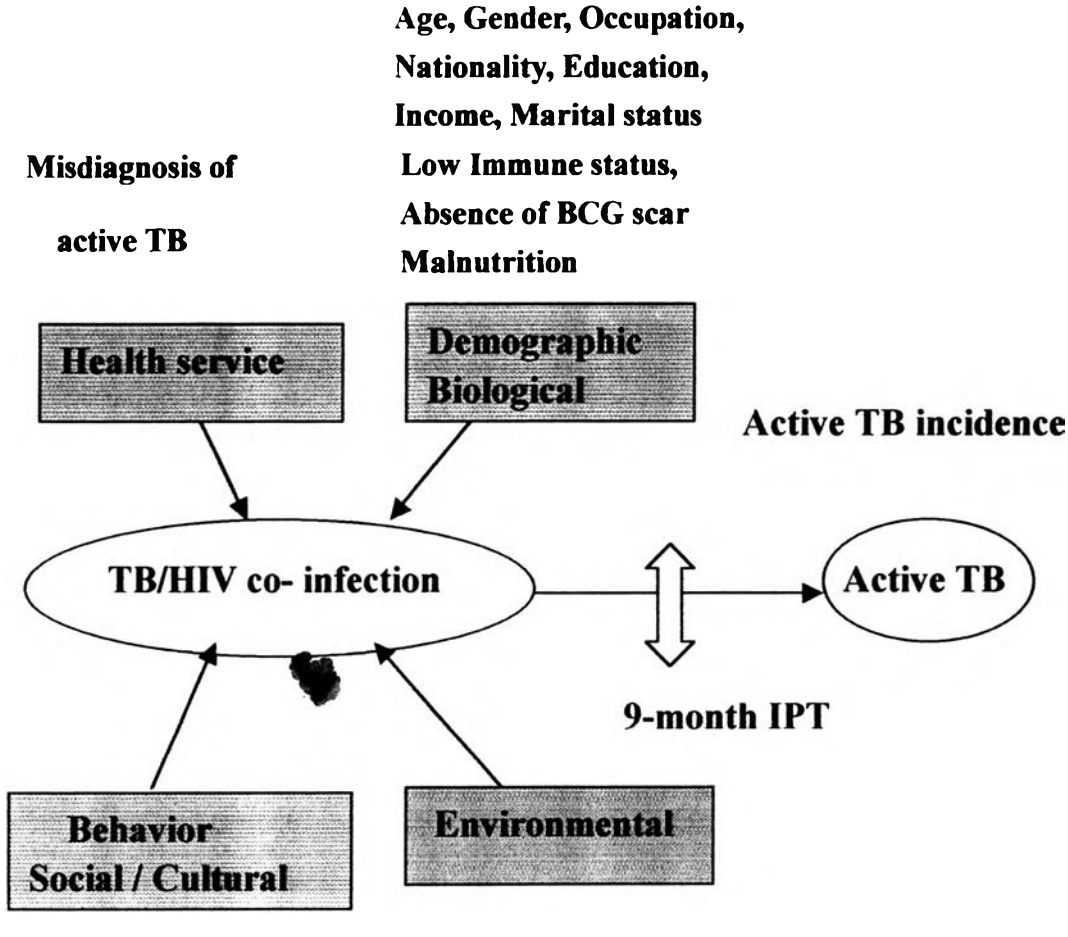
- Below15 and over 50 years of age
- Current active TB
- Past history of active TB
- Pregnancy.
- Abnormal liver enzymes. (Aspartate aminotransferase > 122 U/L)
- Serious illness.

* Those with antiretroviral therapy will not be excluded.

3.8. Conceptual framework

IPT protects the development of active TB among TB/ HIV co-infected persons but some people develop active TB in spite of IPT. So this conceptual framework shows the relationship between some factors related to development active TB and protective effect of IPT. (Fig 3.2)

Fig 3.2. Factors affecting the development of active TB



3.9. Dependent (Outcome) Measurement – Active TB

Definition of active TB case

1. Confirmed TB (Pulmonary TB or Extra-pulmonary TB)

Clinical case of TB of the lung, lymph node, abdominal organ, central nervous system, disseminated and other organs with positive acid-fast bacilli (AFB) smear and/or culture.

2. Probable TB

2.1. Chest X-ray consistent with active TB (infiltration or cavity) with negative AFB and culture, excluding suspected case of inactive pulmonary TB with old inactive infiltration or cavity

2.2. Clinically diagnosed active extrapulmonary TB based on clinical evidence, e.g. cervical lymph node swelling, abdominal pain with fever, abnormal symptoms of central nervous system including lost or blurred consciousness, without positive AFB or culture.

* For patients for whom there is no microbiological confirmations (Probable TB), a

clinical response to antituberculosis medications or histological demonstration of granulomas are required.

TB diagnosis methods: Symptom, Physical examination, Sputum smear, Culture, Chest X-ray

3.10 Independent Variable (Potential Factors)

Potential factors related to development active TB is divided by five components. I. Demographic factors, II. Biological factors, III. Predisposing factors for TB infection, IV. Behavioral factors, V. Factors of provider.

I. Demographic factors:

1. Age: Over 30 years old is likely to develop active TB than younger age.

2. Gender: From the findings of Data Exercises in Chapter IV, male related to low Isoniazid compliance. So, active TB incidence among male might be high.

3. Marital state: Married persons might relate to regular Isoniazid pill taking.

4. City of residence: Some hospitals have good support system for HIV infected people and incidence of active TB might be different in each city.

5. Ethnicity: Divided by Thai (living in Chiang Rai or other province), hill tribe, and foreigner. Foreign ethnicity might relate to low Isoniazid compliance.

6. Occupation: Irregular employment might relate to high active TB incidence.

7. Family income: Low income might relate to high active TB incidence

8. Education level: Low educational level might relate to high active TB incidence.

II. Biological factors

1. Body mass index (BMI= weight in Kg/height ^{2}): Low body weight is a risk factor for the development active TB.

2. Complete blood cell count (Lymphocyte count, Hemoglobin): Lymphocytes count of 2 X10 ^{9}/l or higher or hemoglobin of 10 g/dl or higher related to higher effect of IPT.

3. Percentage CD4 lymphocyte count: Percentage CD4 lymphocytes reflect the function of immune system and low percentage related to high active TB incidence.

4. Anergy to skin test: Mumps antigen negative reaction means anergy. This condition reflects low immune system and become risk factor of active TB.

5. **No BCG vaccine scar:** BCG reduces the risk of TB about 50 %. So, no BCG scar becomes risk factor active TB.

6.**Development of AIDS symptom or opportunistic infection:** Symptoms of AIDS and opportunistic infection reflect low immune system and become risk factor.

7. **Presence of adverse reaction of Isoniazid:** adverse reaction induces low compliance of Isoniazid and become risk of active TB.

8. **Use of antiretroviral drugs:** This drug reduces the progression of HIV disease and protects active TB

9. **Use of antiopportunistic infection prophylaxs:** The history of using this drug reflects low immune system and become risk factor.

III. Predisposing factors for TB infection:

1. **Daily alcohol drink:** Alcoholism might become risk factor.

2.**History of cigarette smoking:** This condition related to lung disease and become risk factor.

3. **History of imprisonment:** Casado J. L.et al. (2000) reported that this situation increases the exposure of active TB and become risk factor.

4.**Homelessness:** This situation related to malnutrition and exposure of active TB and

become risk factor.

5. **Continuation of active intravenous drug use:** From the study in US, incidence of active TB among drug users co-infected with HIV and TB is high. (76 cases per 1000 person-years). (CDC MMWR 2000)

6. **The past history of Lung Disease:** Relative risk of active TB is 30 in silicosis compared to health persons. (CDC MMWR 2000)

7. **The presence of Diabetes mellitus:** Relative risk of active TB among Diabetes mellitus patients reported 2-4. (CDC MMWR 2000)

8. **The presence of chronic disease:** Relative risk of active TB is 27-63 in jejunoileal bypass, 16 in cancer of head or neck.

9. **Intimate contact with a patient with active TB:** Casado J.L.et al. (2000) reported that exposure of TB is risk factor.

IV. Behavioral factors:

1. **The number of Isoniazid to be taken during 9-month IPT:** High compliance of Isoniazid during 9 months might relate to low incidence of active TB.

2. **The number of Isoniazid to be taken after 9-month IPT:** High number of Isoniazid to be taken after 9 months means participants cannot finish 270 Isoniazid

pills during 9 month. This situation might relate to high incidence of active TB.

V. Factors of provider

1. **Number of hospital visits during IPT:** Low number of hospital visit might reflect low compliance of Isoniazid.
2. **Distance from hospital:** Relative risk of IPT default is reported 0.67 among IPT participants living in the same district of the hospital (Piyaworawong, 2000). Long distance from hospitals maybe related to high incidence of active TB.
3. **The method for going to the hospital:** The incidence of active TB between persons using public transportation and own car might be different.
4. **Entering the daycare program:** Relative risk of IPT default is reported 0.57 among HIV infected person entering day care activity (Piyaworawong, 2000). So maybe entering the daycare program relate to protective effect of active TB.

3. 11 Sample size

The sample size was calculated for gathering objective 4; Factors affecting the development of active TB among HIV infected persons who completed 9-month IPT. The factors that selected for sample size calculation are two types of active TB

screening method. Screening A is using symptoms and signs, sputum smear, culture and chest X-ray. Screening B is using only symptoms and signs and sputum smear. The proposed sample size was calculated to detect the difference of the incidence of active TB between IPT participants with screening A and screening B.

The incidence of active TB was based on the report of Fitzgerald D W et al. (2000). The study in Haiti, of 1005 tuberculin skin test positive HIV infected persons who completed Isoniazid, 14 (1.4%) subsequently had active TB diagnosed. These HIV infected persons were screened for active TB by using screening A.

The sample size determination is calculated using a formula below. (Fleiss, 1981)

$$n = \sqrt{Z_{\alpha/2}\sqrt{(R+1)PQ} - Z_{1-\beta}\sqrt{(RP_1Q_1+P_2Q_2)}}$$

$$N = .25\, n \times \sqrt{1+\sqrt{1+2 \times (R+1)}} / [nR \times |(P_2-P_1)|]$$

n = Sample size from the first population

Rn = sample size from the second population. Total sample size is (R + 1) n.

P = true proportion of factor in the population (guess)

Q = 1 – P

This sample size calculation is based on the assumption that 80 % of IPT participants will receive active TB screening A and 20 % of IPT participants will receive active TB screening B. Because the information collected through a Data Exercise in Chiang Rai (Chapter IV) reveals that some IPT participants did not be checked chest X-ray or culture before enrolled IPT. So some IPT participants might not receive chest X-ray or culture during the prospective cohort study.

Estimated active TB incidence among participants who will receive active TB screening A = 1 %

Expected active TB incidence among participants who will receive active TB screening B = 4 %

Ratios of participants who will receive screening A : screening B = 4 : 1

Type I error = 0.5

Type II error = 0.2

The proposed sample size was calculated based on 95% confidence level and 80% power using EPI info version 6.02. Sample size of participants with active TB screening A need to be 1040 and participants with active TB screening B will need to be 260. Therefore, the total sample size needed is 1300.

3.12 Data collection procedure

3.12.1. All IPT registered cases

The information described data collecting forms (Appendix I - IV) will be measured through face-to-face interviews by the research team at the time when participants visit the outpatient clinic or day care monthly meeting. Also medical records and IPT registration in each hospital will be investigated periodically.

3.12.2. Investigation of active TB cases among IPT participants

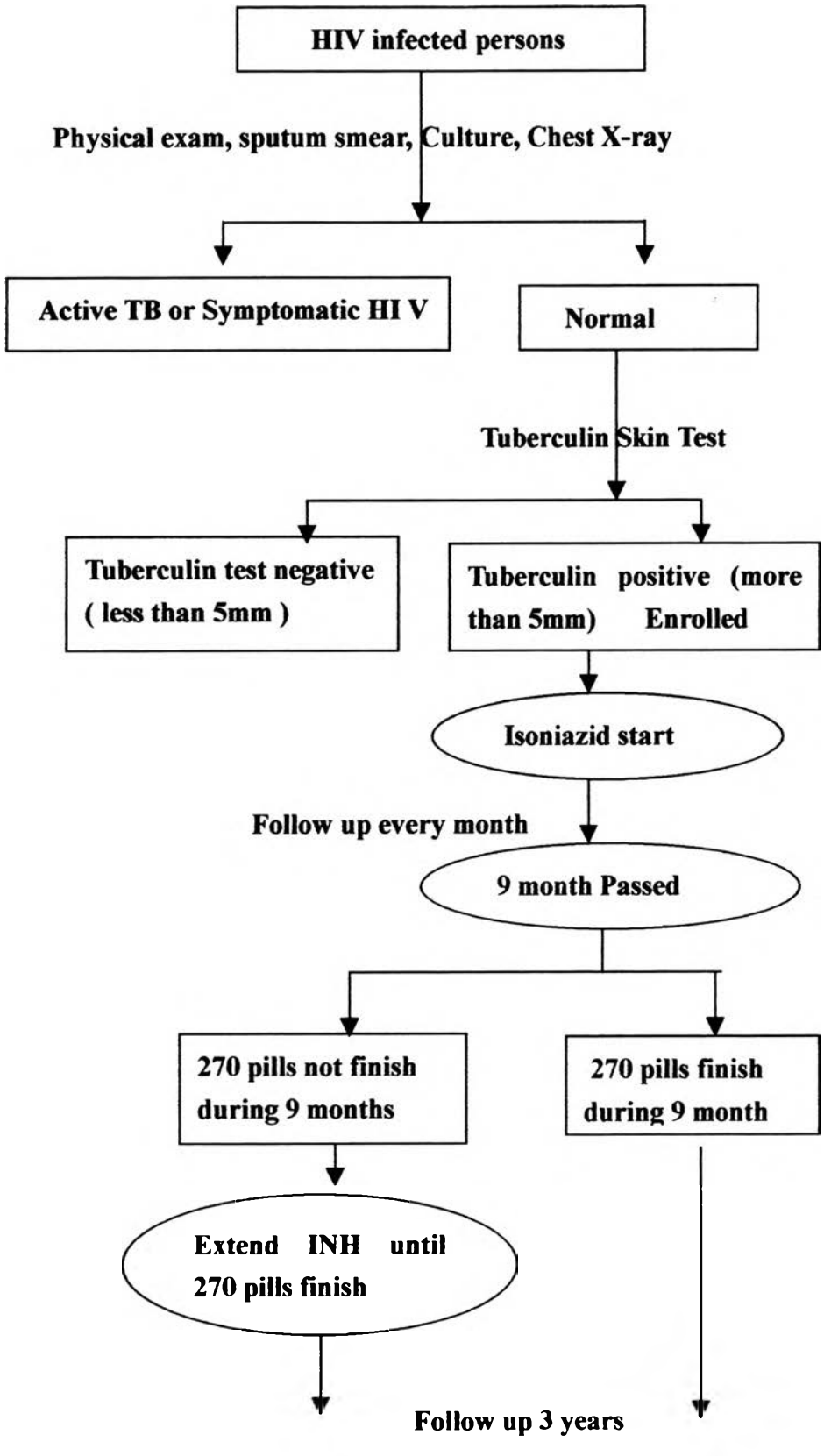
Active TB cases among HIV infected persons with IPT will be registered in the TB registration at each hospital. TB information about symptoms before diagnosed active TB, time from starting IPT to become active TB, CD4 cell count, a location of TB, the result of sputum smear, culture, chest X-ray, drug sensitivity test using sputum TB bacteria and TB treatment outcome will be collected by face-to-face interviews and

observation of medical records and TB registration using TB case investigation form (Appendix V).

3.12.3 Steps of data collection

The steps of data collection were described as a flow chart. (Fig 3.3)

Fig 3.3. Data collection flow chart



Data will be collected using these 5 forms

1. IPT screening List. (Appendix I)
2. IPT enrollment form. (Appendix II)
3. IPT follow up form for during Isoniazid therapy. (Appendix III)
4. IPT follow up form after Isoniazid therapy. (Appendix IV)
5. TB case investigation form. (Appendix V)

3.12.4 Withdrawal from the study after enrollment

Criteria for withdrawal will be 1) The participant's voluntary withdrawal, 2) Development of active TB, 3) Move to another province with difficult to access to the study hospital, 4) The study clinician's discretion for medical complications of AIDS or suspected Isoniazid side effects.

3.13 Research activity planning

3.13.1. Set up research program

Discuss with ethical committee about research proposal and obtain approval from the Thai Ministry of public health. After that, inform the hospitals the objectives and

guidelines of the study and discuss with each hospital.

Making the research team and providing training about interviewing and filling in data collecting forms.

Provide training of IPT counseling and study methods for hospital staffs.

3.13.2. Enrollment

The programme will recruit newly diagnosed HIV infected persons from the post HIV test-counseling clinic There is a data that total 5702 persons diagnosed HIV infection during 5 years in Chiang Rai. So, during 3 months enrollment, about 300 persons might be newly diagnosed HIV infection.

Also already known HIV infected persons without history of IPT will be recruited from day care activity groups and outpatient clinic. From the data of 5702 HIV infected persons collected from 1995, 1276 cases have already died and 1766 cases have already received IPT. So, there are 2660 HIV infected persons who have no history of IPT in Chiang Rai. These cases have possibility to join to this study. So this study can invite participant to IPT from about 3000 HIV infected persons.

Potential participants will be told about the objective of the study and if they agree to participate in this study, they will be interviewed about present and past disease history and current symptoms of TB and AIDS by research nurses using the IPT screening list (Appendix I). After that, potential participants will provide a on the spot sputum sample for smear test and given a sputum container to take home for an early morning sample. They also will be injected tuberculin skin test by research nurses. These research nurse will be received training and have enough experience in performing tuberculin skin test and reading the result.

For checking tuberculin skin reaction, potential participants will come back to hospital after 48 to 72 hours after injection. The same day, they will bring sputum, which will be collected in the early morning for the second times sputum smears test and culture. The positive tuberculin skin reaction will be defined as an diameter of indurations become over 5 mm. Participant with positive tuberculin skin test will receive physical examination, chest X-ray and they will be asked to provide on the spot sputum sample for the third times sputum smear test and the second times culture. Sputum smear and chest X-ray will be performed in each hospital, but culture test will be performed in Chiang Rai hospital.

If there is no active TB and exclusion criteria, potential participants will be invited to join IPT. Participants will provide written consent when they are enrolled.

Every week, number of IPT enrollmentwill be informed to main office and the total number will be calculated. The duration of enrollment is expected for 3 months, but enrollment will be continued until 1300 sample size will be reached.

3.13.3. Investigation of newly registered IPT participants.

For participants with newly registered IPT, data of height, body weight, presence of BCG scar, blood test for complete blood cell count, liver function test and CD 4 lymphocyte count will be collected by the research nurse and will be filled in the IPT enrollment form. (Appendix II)

3.13.4. Follow up the cohort and measuring outcome

3.13.4.1 During 9-month IPT

Participants will be provided with daily self-administered 300 mg dose of Isoniazid, and 10 mg daily Piridoxine (vitamin B complex) for 9 months. During prescribed

Isoniazid, participants will be followed up every month in the day care monthly meeting or out patient's clinics. They will be interviewed about compliance, side effects of Isoniazid, symptom of active TB, clinical progression of HIV disease by research staffs using IPT follow up form for during Isoniazid therapy. (Appendix III)

3.13.4.1.1 Follow up for active TB

If participants develop any symptoms related to TB, the participant will be told to halt Isoniazid (But continue Vitamine B). They will take physical check by physician and undergo chest X-ray, three times sputum smear and sputum culture. If active TB is not diagnosed, they will be instructed to resume Isoniazid and continue in the study.

3.13.4.1.2. Follow up for Isoniazid compliance

The participants will be required to bring their leftover Isoniazid medicine for pill count on a monthly basis and reasons for discrepancies will be explored. At every follow-up visit, participants will be encouraged to improve compliance.

3.13.4.1.3 Follow up for Isoniazid drug toxicity

Drug toxicity will be evaluated by the research nurses at monthly follow up and study doctors at any unscheduled visit for illness. Also complete blood cell count and liver

function tests will be carried out at 1, 3, 6, 9 month after starting Isoniazid. If any symptoms, which suspected drug toxicity are observed, participants will be assessed by history, physical examination and laboratory evaluations. If liver function tests shows an increase 3 times the upper limits of normal values they will take off medication while repeat tests will be done.

3.13.4.1.4. Follow up for clinical progression of HIV disease

Clinical progression of HIV disease is defined as the first occurrence of an acquired immunodeficiency syndrome (AIDS).

3.13.4.2. After 9-month IPT

When 9-month IPT is finished, IPT outcome will be defined as

1. Complete

   -When 9-month IPT finish, the participants can be followed-up.

2. Defaulted

   - Participants interrupted Isoniazid for 2 consecutive months or more.

3. Transfer out

   - Participants referred to receive treatment at another place and IPT

outcome is unknown.

4. Died

   - Participants died from any causes during IPT.

5. Severe Isoniazid adverse reaction

6. Developing active TB during IPT

7. Others

If participants do not finish 270 Isoniazid pills at the end of 9 months, they will be encouraged to extend IPT more than 9 months until they will finish taking all pills. Participants who extend IPT more than 9 months will be followed-up every month during prescribed Isoniazid.

3.13.4.2.1. Follow up for active TB

All participants who finishes taking 270 Isoniazid pills will be followed up at least every 6 months after 9-month IPT. But if participants entered day care activities, active TB and AIDS symptoms will be evaluated by the research nurses during monthly daycare meeting. Every 12 months, complete blood cell counts and CD4 count and chest X-rays will be checked and research nurses will fill out IPT follow up form after Isoniazid thearpy. (Appendix IV)

If participants develop any symptoms related to active TB, the participant will take physical check for illness by study physicians at any unscheduled visit and undergo chest X-ray, three times sputum smear and culture. If active TB is not diagnosed, they will continue in the study.

The medical records, ITP and TB registration in each hospital, provincial-wide computerized TB registration and mortality database in Provincial Health Office will be checked periodically by research staffs.

3.13.4.3. Loss to follow up

To minimize loss of follow-up, at the time of enrollment, participants will be asked to provide name, telephone number, and address of a friend or family member who will likely contact with the participants. If participants will 4 weeks late for a follow-up visit during prescribed Isoniazid or no visit for 3 month after finishing Isoniazid, research nurse will attempt to locate the participants by calling or visiting participant's home and encourage them to return to the hospital.

3.14 Laboratory and diagnostic testing

HIV test: Two positive ELISA (Enzime-linked immuno-sorbent assay) or rapid tests are considered positive for HIV infection. Two negative ELISA or rapid tests are considered negative for HIV infection. Discordant results by ELISA are confirmed by a third ELISA. Discordant results by rapid test are confirmed by double ELISA.

Tuberculin skin test: Potential IPT participants will be given tuberculin skin test (TST) by the Mantoux method, with 5 tuberculin units (0.1 ml) of commercial purified protein derivative of tuberculin (PPD-tuberculin) in the volar surface of forearm. The result will be read 48 to 72 hours later.

Acid-fast bacilli (AFB) sputum smears: AFB smears will be performed in each hospital. Process in laboratory room is drying, smearing staining with a basic fuchsin dye (Ziehl-Neelsenmethod) and examined by microscope

Mycobacterial culture: Specimen of Culture will be send to laboratory room in Chiang Rai hospital by research staffs. The first process of the test, specimens will be liquefied and decontaminated before culturing. The second process is, to isolate

mycobacteria from a processed specimen, 0.1-ml quantities are inoculated onto slants of solid medium and incubated at 35-37 ℃ under 5% co2 for 3 weeks.

Antibiotic sensitivity of *M. tuberculosis*: The susceptibility to Isoniazid, Rifampicin, Streptomycin, and Ethambutol will be performed at the TB division in Bangkok

Chest X-ray: Posteroanterior chest X-ray will be done in each hospital. The chest X-ray will be interpreted by a physician.

CD 4 counts testing: Enumeration of CD4+ T- lymphocyte cell counts will be performed in Chiang Rai hospital.

Blood test (Complete blood cell count, Liver function): Hemoglobin, hematocrit, platelet, and white blood cell counts for complete blood cell count and Total -Bil, ALT, AST for liver function test will be done according to the clinic routine.

3.15 Data processing and analysis

- Data will be transcribed Epi-info version 6 (US Centers for disease control and prevention)
- Incidence rate using cumulative incidence and incidence density will be calculated
- Univariate analysis: Relative risk with 95 % confidence interval will be performed to determine the strength of association between factors and outcome event (active TB).

The formula of cumulative incidence and incidence density described below.

$$\text{Cumulative Incidence} = \frac{\text{new cases of a disease during a specified period}}{\text{Population at risk (PAR)}} \times 10^{n}$$

$$\text{Incidence density} = \frac{\text{new cases of a disease during a specified period}}{\text{Person-years of observation of PAR}} \times 10^{n}$$

(WHO, 1992)

3.16 Research plan

Table 3.4 Research plan time table

Activities	2002								2003	2004	2005	2006					
	1 - 5	6	7	8	9	10	11	12				1-4	6	7	8	9	10
Develop proposal	→																
Submission to ethical committee in Ministry of Public Health		→															
Making & training research team			→														
Further agreements with the research team and local coordinators				→													
data collection				→											→		
Preliminary data analysis													→			→	
data analysis and interpretation																→	→
Report writing																	→

3.17 Ethical Considerations

The ethical considerations regarding involvement of human subjects described are:

1.New risks and benefits to the participants.

2. Consent procedures.

3. Participant confidentiality.

3.17.1 Balance of Risks and Benefits

Participants will benefit in this study from a more active TB screening and follow-up than usual IPT participants. Also, participants will be given active TB knowledge and education, which may lead lower subsequent risk and earlier detection of active TB. Those who have active TB signs and symptoms will be consulting the physician for active TB screening immediately.

The risks involve the inconvenience of participating in the study, risks related to tuberculin skin tests, side effects of Isoniazid and blood draw (minor discomfort, hematoma, and infection at the bleeding site.)

Regarding questionnaires, if the participants feel uncomfortable answering some of the questions, they have a right to skip or stop answering. Also to protect the risk related to skin tests, including hypersensitivity leading to large reaction with ulceration and sloughs at the injected site and contaminate infection, the internationally recognized standardized antigens will be used; these are already widely used and proven as safe. Also participants who have allergies to eggs or thimerosal will not receive the mumps

antigen. In addition, well-trained personnel will carefully perform the skin tests to minimize the pain at the injection sites and monitor the risk mentioned above.

Regarding risk related to Isoniazid, to detect side effects earlier, every month research nurses will screen side effects and counsel for participants on self-skills to find side effects.

Risks will be protected carefully and balance of risks and benefits is acceptable.

3.17.2. Ethical approval

Research protocol will be sent to the Ethical Committee, Ministry of Public Health, Thailand.

3.17.3. Consent Procedures

Expected participants will be informed about content of IPT and the objective of this study and participants have freedom to participate. If they agree to participate in the study, written informed consent will be obtained in the Thai language. If participants are not able to understand Thai language, consent will be obtained using interpretation.

3.17.4. Confidentiality

Participant's specific numerical identification number will be the only personal identifier in the computerized password-protection database. All documents will be stored in a secure place. Informed consent with both name and numerical identification number will be destroyed after the data analysis is finished. Only the person in charge of the analysis will have access to the computer file.

3.18. Expected outcome

1. The data of 9 month and 3 year incidence of active TB among HIV infected persons registered for 9-month IPT will be used for evaluation of short and long-term impact of IPT.

2. Identified factors associated to development of active TB among HIV infected persons registered for 9-month IPT will be applied for improvement of IPT guideline.

3.19 Budget Plan (For 45 months)

Total budget of this proposal is 8,704,000 Baht. (Table 3.5) The researcher will apply to get foundation for some international or Japanese organizations which supporting TB or HIV research project.

Table 3.5. Budget plan

Item	Description	Amount
		baht
1	Salary	
	1.1 Salary for 5 research assistance (10,000 baht * 45 months)	2,250,000
	1.2 Salary for 1 Research Coordinator (20,000 baht * 45 months)	900,000
2	Medical supplies	
	2.1 Cost of skin test antigen	
	2.1.1 Tuberculin (1300 test * 35 baht)	45,500
	2.2.2 MUMPS (1300 test * 150 baht)	195,000
	2.2 Cost of needle and syringe for skin test (2700 test * 5 baht)	13,500
	2.3 Cost of chest x-ray (120 baht * 3000 cases)	360,000
	2.4 Sputum examination (60 baht * 1500 specimens)	90,000
	2.5 *Mycobacterium* culture (100 baht * 4000 specimens)	400,000
	2.6 Blood liver function test (200 baht * 6000 specimens)	1,200,000
	2.7. Complete blood cell count (50 baht * 8000 specimens)	450,000
	2.8. CD4 lymphocyte count (800 baht * 2600 specimens)	2,080,000
3	Transportation	
	3.1 Sputum culture to Chiang Rai hospital (50 baht * 2600 cases)	130,000
	3.2. Drug registrant test to TB division (100 Baht * 2600 cases)	260,000
4	Report Printing	30,000
5	Miscellaneous (Xerox, communication, etc)	300,000
	Total	**8,704,000**

References

Akarasewi P, et al, (1999). Risk of active TB in HIV infected persons following 9 months Isoniazid preventive therapy. IUATLD, vol 3, Number 9, S23.

Bucher HC, Griffith LE, Guyatt GH et al. (1999), AIDS.13: P501-507

Communicable disease control 10 in Chiang Mai. (2000). Isoniazid Preventive Therapy for People With HIV in the Upper North of Thailand.

Casado J L et.al. (2002). Clinical Infect Dis Feb 1, 34(3): P 386-9.

CDC (1995). Self study modules on Tuberculosis.

CDC (2000). MMWR. Targeted tuberculin testing and treatment of latent tuberculosis infection. June 9,vol. 49,No. RR-6.

Fitzgerald D W et al. (2000). Active tuberculosis in individuals infected with human immunodeficiency virus after Isoniazid prophylaxis. Clin Infect Dis. (6) P1495-7.

Fleiss. (1981). Statistical methods for rates and proportions. 2nd ed., Wiley, P 38-45.

Mwinga A. et al. (1998). Twice weekly tuberculosis preventive therapy IsoniazidIV infection in Zambia. AIDS, 12: P 224-2457.

Ministry of Public Health (2000). Thailand Health Profile 1997-1998.

Moreno S. et al. (1997). Isoniazid Preventive Therapy in Human Immunodeficiency

Virus-Infected Persons. Arch Intern Med. 157, P1729 – 1734.

Piyaworawong S et al. (2000). Tuberculosis preventive therapy as part of a day care package for people living with HIV in a district of Thailand. AIDS,15 (13) P1739-41.

Siriarayapon. P. (2000). Impact of HIV on Tuberculosis in Northern Thailand. Thesis submitted for the degree of Doctor of Public Health, University of London.

UNAIDS. (2000). Secretariat joints STOP TB initiatives, stress close links between HIV and Tuberculosis.

WHO and UNAIDS. (1998). Policy statement on preventive therapy against TB in people living with HIV.

WHO. (1992). Health research methodology, P52.

WHO. (1997). Guidelines for national programmes.