FACTORS ASSOCIATED WITH TUBERCULIN SKIN TEST REACTIVITY AMONG HIV-INFECTED PEOPLE IN BANGKOK

Narin Hiransuthikul[1], Mattana Hanvanich[2], Gregory J Dore[3], Thawatchai Mokkhawes[4], Yueming Li[3], Jos Perriens[5] and John M Kaldor[3]

[1]Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok; [2]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [3]National Center in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia; [4]Program on AIDS, Thai Red Cross, Bangkok, Thailand; [5]UNAIDS, Geneva, Switzerland

Abstract. INH preventive therapy (IPT) has been shown in several randomized controlled trials to reduce the risk of developing active TB in tuberculin skin test (TST) or purified protein derivative (PPD) positive HIV infected individuals. Detection of latent tuberculosis by TST and determination of factors associated with the PPD positivity in HIV-infected persons are important for the targeting of chemoprophylaxis. Six hundred asymptomatic and early symptomatic HIV-infected subjects attending the AIDS Clinic of the Chulalongkorn University Hospital, Bangkok, Thailand were enrolled in two randomized clinical trials of chemoprophylaxis against TB from December 1994 to December 1996. The availability of baseline characteristics, including TST reactivity, among these participants enabled a cross-sectional analysis of factors associated with PPD positivity. The results showed that 117 (19.5%) were PPD positive and 483 (80.5%) were PPD negative with ages 18-65 years (median 29 years). HIV exposure category was 46.2%, 34.5%, and 6.7% for heterosexual contact, commercial sex work, and homosexual and bisexual male contact respectively. The median CD4 cell count was 315/mm^3 (range, 5-1,074/mm^3). HIV exposure category and CD4 cell count were significantly associated with PPD status. Homosexual/bisexual contact had 3 times higher risk of PPD positivity than heterosexual contact (adjusted OR=2.9; 95% CI,1.4-6.1) and risk of PPD positivity was higher among patients with CD4 cell counts of 200-500/mm^3 (adjusted OR=1.8; 95% CI,1.0-3.1) and above 500/mm^3 (adjusted OR=3.4; 95% CI,1.7-6.7) when compared to patients with CD4 cell counts of less than 200/mm^3. The HIV-infected persons in Bangkok with homosexual/bisexual contact are at higher risk for latent TB. Population-based tuberculin screening without accompanying HIV testing cannot be used to estimate the prevalence of actual latent TB in a population where HIV infection is widespread, such as in Thailand.

INTRODUCTION

Most HIV-infected persons with latent tuberculosis have a greater probability of reactivation and developing active tuberculosis due to impaired cell-mediated immunity (Narain *et al,* 1992; Giradi *et al,* 2000). In addition, HIV-infected persons are at increased risk of primary progressive tuberculosis which can develop within weeks after infection (Daley *et al,* 1992). In several regions where the background prevalence of tuberculosis is high, the introduction of HIV infection has led to dramatic increases in the incidence of tuberculosis. In northern Thailand, a declining incidence of tuberculosis during the 1980s was transformed by the HIV epidemic into a rapid rise in the incidence during the 1990s (Yanai *et al,* 1996). The WHO has estimated that in 1990 there were over 300,000 tuberculosis cases attributable to HIV infection, and this number was anticipated to increase to 1,400,000 cases in the year 2000 with more than half living in Africa and Southeast Asia (Dolin *et al,* 1994). In Thailand the prevalence of coexisting HIV infection in new tuberculosis patients has increased steadily from 3.8% in 1991 to 22.3% in 1996 (Payanandana, 1993; 1995; Anonymous, 1999).

The risk of tuberculosis in persons with HIV infection varies according to tuberculin or purified protein derivative (PPD)-skin test status. For PPD positive persons the estimated annual risk is 5.5-16.2% and for PPD negative persons the risk is estimated at 0.0-8.1% for anergic persons and 0.0-

Correspondence: Dr Narin Hiransuthikul, Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

4.5% for persons with intact cutaneous hypersensitivity (Selwyn *et al*, 1989; 1992; Braun *et al*, 1991; Allen *et al*, 1992; Wadhawan *et al*, 1992; Guelar *et al*, 1993; Moreno *et al*, 1993; Pape *et al*, 1993; Antonucci *et al*, 1995). These estimates are in sharp contrast with those among HIV-negative PPD positive persons whose annual risk of developing tuberculosis is less than 0.2% (Styblo, 1991).

A recent meta-analysis of randomized placebo-controlled trials of isoniazid preventive therapy among people with HIV infection demonstrated an approximately 70% reduction in incidence of tuberculosis and a 27% reduction in mortality among PPD positive subjects (Wilkinson *et al*, 1998) . In contrast, there was no significant reduction in tuberculosis incidence or mortality among PPD negative subjects (Wilkinson *et al*, 1998). Thus, the detection of latent tuberculosis in HIV infected persons is crucial for the targeting of chemoprophylaxis. As the tuberculin skin test remains the standard, simplest and most frequently used technique in detection of latent tuberculosis, determination of factors associated with the PPD positivity among persons with HIV infection is a priority. The availability of baseline characteristics, including PPD reactivity, among a large number of HIV-infected persons enrolled in two randomized clinical trials of tuberculosis chemoprophylaxis in Bangkok enabled a cross-sectional analysis of factors associated with PPD positivity.

MATERIALS AND METHODS

Enrollment of study participants

In December 1994, enrollment in two randomized trials of chemoprophylaxis against tuberculosis commenced in Bangkok. The trials consisted of (1) a comparison between twelve months of isoniazid and placebo among PPD negative HIV-infected persons, (2) a comparison between twelve months of isoniazid and four months of isoniazid plus rifampicin among PPD positive HIV-infected persons. Study participants were voluntarily recruited from asymptomatic or early symptomatic HIV infected subjects attending the AIDS Clinic of the Chulalongkorn University Hospital, Bangkok.

Inclusion criteria included adults greater than 15 years, serological evidence of HIV infection (documented by ELISA with confirmation by a second ELISA or a rapid test), no evidence of pulmonary tuberculosis (negative chest radiography for active tuberculosis plus negative sputum microscopic examination for acid-fast bacilli (AFB) when cough and expectoration were present), no evidence of extra-pulmonary tuberculosis (absence of fever, weight loss, nocturnal transpiration, asymmetric lymphadenopathy and intra-thoracic lymphadenopathy on chest radiography), no evidence of a life threatening opportunistic infection of malignancy, a Karnofsky score of 70 or more and written informed consent.

Exclusion criteria included a diagnosis of clinical tuberculosis prior to the start of preventive therapy, a prior history of tuberculosis therapy or preventive therapy defined as 7 or more days of any anti-mycobacterial therapy, liver disease as evidenced by clinically apparent jaundice and known hypersensitivity to any of the study drugs and, for the PPD positive group, concomitant therapy with other drugs whose metabolism may be accelerated by rifampicin.

A total of 600 HIV-infected persons were enrolled up to September 1996 when enrollment ceased for both trials. Baseline data included demographic characteristics (age, sex, birthplace, educational level and income), HIV exposure, PPD skin test reactivity and CD4 cell count.

Skin test

Demographic characteristics and HIV exposure were obtained through a standardized interview. The PPD skin test was performed using the Mantoux technique by an intra-dermal injection 0.1 ml of PPD-S (5IU) on the volar surface of the forearm by trained nurses. The diameter of induration was measured in two perpendicular directions by the pen and palpation method and the average diameter was recorded. To assess anergy, a MULTITEST was performed with seven skin test antigens: tetanus, diptheria, streptococcus, tuberculin, candida, tricophyton, and proteus. All skin tests were read by the trained nurses within 48-72 hours after the intra-dermal injection. The interpretation of the test was according to the recommendation by WHO and the Center for Disease Control (CDC) (CDC, 1989; WHO, 1989; Arnadottir, 1996). The reaction was considered PPD positive if the induration was 5 mm or more in diameter. Patients were considered anergic if the combined MULTITEST reading was 5 mm or less. The CD4 cell count was measured by flow cytometry using FACSORT (Beckton-Dickinson, San-Jose, California).

Table 1
Factors associated with PPD positivity among trial subjects (n=600).

Variable	N (%)	PPD+ve	%PPD+ve	p-value[a]	OR[b] (95%CI)	p-value[b]
Age (years)				0.50		
<29	306(51.0)	64	20.9			
30-39	223(37.2)	38	17.0			
40+	71(11.8)	15	21.1			
Sex				0.29		
Female	231(38.5)	40	17.3			
Male	369(61.5)	77	20.9			
Educational level				0.22		
Primary or less	168(28.0)	29	17.3			
Secondary or vocational	229(38.2)	52	22.7			
Higher	143(23.8)	29	20.3			
Unknown	60(10.0)	7	11.7			
Birthplace				0.51		
Bangkok	140(23.3)	31	22.1			
Central	149(24.8)	27	18.1			
North and Northeastern	217(36.2)	45	20.7			
Other	94(15.7)	14	14.9			
Income (bath/month)				0.03		
<2,500	73(12.2)	20	27.4		1.0	
2,500-4,999	101(16.8)	11	10.9		0.34(0.15-0.78)	0.01
5,000-9,999	208(34.7)	48	23.1		0.80(0.43-1.50)	0.49
>10,000	151(25.2)	29	19.2		0.60(0.30-1.19)	0.15
Unknown	67(11.2)	9	13.4		0.23(0.06-0.81)	0.02
HIV exposure category				0.02		
Heterosexual	277(46.2)	47	17.0		1.00	
Commercial sex worker	207(34.5)	40	19.3		1.44(0.87-2.37)	0.16
Homosexual/bisexual	40(6.7)	15	37.5		2.94(1.41-6.11)	0.004
Other/unknown[c]	76(12.7)	15	24.6		3.16(1.13-8.82)	0.03
CD4 cell count (/mm^3)				0.001		
<200	160(26.7)	18	11.2		1.00	
200-500	344(57.3)	69	20.1		1.79(1.01-3.14)	0.04
>500	96(16.0)	30	31.2		3.41(1.72-6.69)	<0.0005

[a]Chi-square test; [b]Adjusted odds ratio (logistic regression); [c]Intravenous drug use (12), blood transfusion (4) and missing data (60).

Data analysis

Chi-square test was used to assess associations between PPD skin test reactivity and demographic characteristics, HIV exposure category and CD4 cell count (<200/mm^3, 200-500/mm^3). Independent factors were then determined using logistic regression. The initial analyses were conducted on the total study population (n=600). As a proportion of HIV-infected persons who test PPD negative do so because of immunosuppression-related skin test anergy, analyses were repeated excluding anergic patients (n=136).

RESULTS

From December 1994 to September 1996, 600 asymptomatic and early asymptomatic HIV infected patients had a PPD skin testing prior to enrollment in the two randomized clinical trials. Of these, 117 (19.5%) were PPD positive and 483 (80.5%) were PPD negative. Demographic characteristics are shown in Table 1. Patients were ages 18-65 years (median 29 years), and the male to female ratio was 1.6:1. The level of education of most subjects were secondary or vocational level (38.1%) and primary level or less (28.0%). North and northeast Thai-

land was the most common birthplace (36.2%), with smaller proportions born in Bangkok (23.3%) and central Thailand (24.8%).

The HIV exposure category was heterosexual contact for 46.2% and commercial sex work for 34.5%. Homosexual and bisexual male contact constituted 6.7% of the study population (Table 1).

The median CD4 cell count of the study population was 315/mm^3 (range, 5-1,074/mm^3), with 26.7%, 57.2% and 16.2% in the <200/mm^3, 200-500/mm^3 and >500/mm^3 ranges, respectively (Table 1). Median CD4 cell count was 393/mm^3 among PPD positive patients, 316/mm^3 among PPD negative non-anergic patients and 213/mm^3 among PPD negative anergic patients.

In univariate analysis there were significant associations between PPD status and income (p=0.027), HIV exposure category (p=0.024), and CD4 cell count (p=0.001). Homosexual and bisexual men had the highest prevalence of PPD positivity (37.5%) among HIV exposure categories, while the prevalence of PPD positivity declined with increased levels of immunodeficiency. There were no significant associations between PPD status and age, sex, educational level, and birthplace.

In a multivariate analysis, income, HIV exposure category, and CD4 count remained significantly associated with PPD status. Homosexual/bisexual contact concurred an approximately three times higher risk of PPD positivity than heterosexual contact (OR=2.9; 95% CI 1.4-6.1), and risk of PPD positivity was higher among patients with a CD4 cell count of 200-500/mm^3 (OR=1.8; 95% CI 1.0-3.1) and above 500/mm^3 (OR=3.4; 95% CI 1.7-6.7), when compared to patients whose CD4 cell count was less than 200/mm^3.

A separate analysis was performed excluding patients with anergy (n=136). PPD positivity was 23.7% (110/464) among non-anergic patients, compared to only 2.2% (3/136) among anergic patients. Although income was no longer significantly associated with PPD status, both homosexual/bisexual contact (OR=2.7; 95% CI 1.3-5.6) and high CD4 cell count (> 500/mm^3) (OR=2.6; 95% CI 1.3-5.2) remained associated with PPD positivity in a multivariate analysis among non-anergic patients (Table 2).

DISCUSSION

In our study, demographic factors appeared to have little influence on the rate of PPD positivity in a Bangkok population with HIV infection. Although the lowest income category had the highest prevalence of PPD positivity, there was no significant association with level of income. As expected, the strongest association with PPD status was seen with cell-mediated immune function as measured by the CD4 cell count. However, an additional association was seen with HIV exposure category with homosexual and bisexual men demonstrating a higher rate of PPD positivity.

There were several limitations to our study. Firstly, participants in our study are not necessarily representative of the HIV-infected population in Bangkok. Subjects were selected from persons who attended the AIDS Clinic, Chulalongkorn University Hospital. Secondly, there is potential bias in self report of HIV exposure category. This may be particularly relevant to male to male sexual contact. However, the prevalence of homosexual/bisexual contact among our study population is higher than for HIV or AIDS notification in Thailand (Anonymous, 2000). Thirdly, due to immunodeficiency among people with HIV infection, the sensitivity of PPD skin testing for detection of latent tuberculosis is considerably decreased. Thus, an association with PPD positivity does not necessarily equate to association with latent tuberculosis. On the other hand, true association with latent tuberculosis may be missed due to the lack of PPD positivity. The relatively low overall prevalence of PPD positivity of approximately 20% in our study population is consistent with the majority having a CD4 cell count less than 500/mm^3.

There was a clear association between the PPD positivity and CD4 cell count in our study. Among subjects with advanced immunodeficiency, the prevalence of PPD positivity was 11.2% for those with a CD4 cell count >500/mm^3. PPD positivity depends on intact cell-mediated immune function. A previous Thai study comparing the PPD reactivity between asymptomatic HIV-infected persons and healthy non HIV-infected volunteers in Bangkok showed PPD positivity in 81% of their HIV-infected participants and 89% in their non HIV-infected subjects. However, all of the HIV-infected subjects were asymptomatic with a CD4 cell count 200/mm^3 or above (mean CD4 cell count was 467/mm^3) (Johnson *et al*, 1992; Markowitz *et al*, 1993; Suwanagool *et*

Table 2
Factors associated with PPD positivity among non-anergic trial subjects (n=464).

Variable	N (%)	PPD+ve	%PPD+ve	p-value[a]	OR[b] (95%CI)	p-value[b]
Age (years)				0.71		
<29	233 (50.2)	61	26.2			
30-39	168 (36.2)	38	22.6			
40+	63 (13.6)	15	23.8			
Sex				0.64		
Female	159 (34.3)	37	23.3			
Male	305 (65.7)	77	23.3			
Educational level				0.59		
Primary or less	128 (27.6)	27	21.1			
Secondary or vocational	185 (39.9)	51	27.6			
Higher	118 (25.4)	29	24.6			
Unknown	33 (7.1)	7	21.2			
Birthplace				0.89		
Bangkok	113 (24.4)	30	26.5			
Central	120 (25.9)	27	22.5			
North and Northeastern	170 (36.6)	43	25.3			
Other	61 (13.1)	14	23.0			
Income (bath/month)				0.37		
<2,500	57 (12.3)	18	31.6			
2,500-4,999	65 (14.0)	11	16.9			
5,000-9,999	180 (38.8)	48	26.7			
>10,000	125 (26.9)	29	23.2			
Unknown	37 (8.0)	8	21.6			
HIV exposure category				0.06		
Heterosexual	202 (43.5)	44	21.8		1.00	
Commercial sex worker	177 (38.1)	40	22.6		1.21(0.73-2.00)	0.45
Homosexual/bisexual	37 (8.0)	15	40.5		2.66(1.25-5.61)	0.01
Other/unknown[c]	48 (10.3)	15	31.3		1.84(0.91-3.74)	0.09
CD4 cell count (/mm^3)				0.03		
<200	98 (21.1)	18	18.4		1.00	
200-500	280 (60.3)	66	23.6		1.37(0.76-2.48)	0.29
>500	86 (18.5)	30	34.9		2.57(1.27-5.17)	0.008

[a]Chi-square test; [b]Adjusted odds ratio (logistic regression); [c]Intravenous drug use (12), blood transfusion (4) and missing data (60).

al, 2000). In other studies, the prevalence of PPD positivity in HIV-infected people has varied from 33% to 44% without categorization of the subjects according to the CD4 cell count (Johnson *et al*, 1992; Markowitz *et al*, 1993; Garcia-Garcia *et al*, 2000).

Our study suggests that HIV-infected persons in Bangkok with homosexual/bisexual contact are at higher risk of latent tuberculosis. The explanation for this association is not immediately clear. One possible explanation is social mixing within an environment with a high risk of exposure to active tuberculosis. Our study also supports earlier evidence that population-based tuberculin screening without accompanying HIV testing cannot be used to estimate the prevalence of actual latent tuberculosis in a population where HIV is widespread, such as in Thailand.

ACKNOWLEDGEMENTS

The authors would like to thank Rawadee Paeplod for her assistance in preparing the manuscript. This study was supported in part by the UNAIDS and the National Center in HIV Epidemiology and Clinical Research, University of

New South Wales, Sydney, Australia.

REFERENCES

Allen S, Batungwanayo J, Kerlikowske K, *et al*. Two-year incidence of tuberculosis in cohorts of HIV-infected and uninfected urban Rwandan women. *Am Rev Respir Dis* 1992; 146: 1439-44.

Anonymous. 2nd Review of the National Tuberculosis Programme in Thailand, conducted by a Joint Team of Thailand Ministry of Public Health and WHO, 10-23 July 1999. *WHO/CDS/TB/99. 273, 1999*. 1999.

Anonymous. Number of AIDS patients in Thailand according to risk factors and year of starting illness, September 1984-31st October, 2000.

Antonucci G, Girardi E, Raviglione MC, Ippolito G. Risk factors for tuberculosis in HIV-infected persons: A prospective cohort study. *JAMA* 1995; 274: 143-8.

Arnadottir T, Rieder HL, Trebucq A, Waaler HT. Guidelines for conducting tuberculin skin test surveys in high prevalence countries. *Tuberc Lung Dis* 1996; 77 (supp1): 1-19.

Braun MM, Badi N, Ryder RW, *et al*. A retrospective cohort study of the risk of tuberculosis among women of child-bearing age with HIV infection in Zaire. *Am Rev Respir Dis* 1991; 143: 501-4.

Centers for Disease Control. Prevention and treatment of tuberculosis among patients infected with human immunodefficiency virus: principles of therapy and revised recommendations. *Morbid Mortal Weekly Rep* 1998; 47 (RR-20): 36-41.

Daley CL, Small PM, Schecter GP, *et al*. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length polymorphism. *N Engl J Med* 1992; 326: 231-5.

Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990-2000. *Bull WHO* 1994, 72: 213-20.

Garcia-Garcia ML, Valdespino-Gomez JL, Garcia-Sanchoc, *et al*. Underestimation of *Mycobacterium tuberculosis* infection in HIV-infected subjects using reactivity to tuberculin and anergy panel. *Int J Epidemiol* 2000; 29: 369-75.

Giradi E, Reviglione MC, Antonucci G, Godfrey-Faussett P, Ippoloti G. Impact of the HIV epidemiology on the spread of other diseases: the case of tuberculosis. *AIDS* 2000; 14 (suppl 3): 547-56.

Guelar A, Gatell JM, Verdejo J, *et al*. A prospective study of the risk of tuberculosis among HIV-infected patients. *AIDS* 1993; 7: 1345-9.

Johnson MP, Coberty JS, Clermont HC, *et al*. Tuberculin skin test reactivity among adults infected with human immuuodeficiency virus. *J Infect Dis* 1992; 166: 194-8.

Markowitz N, Hansen NL, Wilcosky TC, *et al*. Tuberculin and anergy testing in HIV- seropositive and HIV-seronegative persons. *Ann Intern Med* 1993; 119: 185-93.

Moreno S, Baraia-Etxaburu J, Bouza E, *et al*. Risk for developing tuberculosis among anergic patients infected with HIV. *Ann Intern Med* 1993; 119: 194-8.

Narain JP, Raviglione MC, Kochi A. HIV-associated tuberculosis in developing countries: epidemiology and strategies for prevention. *Tuberc Lung Dis* 1992; 78: 311-21.

Pape JW, Jean SS, Ho JL, Hafner A, Johnson WD. Effect of isoniazid prophylaxis on incidence of active tuberculosis and progression of HIV infection. *Lancet* 1993; 342: 268-72.

Payanandana V, Bamrungtrakul T, Sriyabhaya K. Report of an internal evaluation of the national tuberculosis programme 1993. Tuberculosis Division, Department of Communicable Disease Control, Ministry of Public Health, Thailand. 1993.

Payanandana V, Kladphuang B, Talkitkul N, Tornec S. Information in preparation for an external review of The National Tuberculosis Programme, Thailand 1995. Tuberculosis Division, Department of Communicable Disease Control, Ministry of Public Health, Thailand. 1995.

Selwyn PA, Hartel D, Lewis VA, *et al*. High risk of active tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med* 1989; 320: 545-50.

Selwyn PA, Sckell BM, Alcabas P, Rriedland GH, Klein RS, Schoenbaum EF. High risk of active tuberculosis in HIV infected drug users with cutaneous anergy. *JAMA* 1992; 268: 504-9.

Styblo K. The impact of HIV infection on the global epidemiology of tuberculosis. *Bull Int Union Tuberc Lung Dis* 1991; 66: 27-36.

Suwanagool S, Chuenarom V, Pechthanon L, *et al*. A comparative study of tuberculin skin test reactivity between asymptomatic HIV-1 seropositive subjects and healthy volunteers. *Asian Pac J Allergy Immunol* 1995; 13: 139-44.

Wadhawan D, Hira SK, Mwansa N, Tembo G, Perrine P. Isoniazid prophylaxis among patients with HIV-1 infection [Abstract TuB 0536]. Amsterdam: Vlll International Conference on AIDS, and lll Sexually Transmitted Disease World Congress, July 1992.

Wilkinson D, Squire SB, Garner P. Effect of preventive treatment for tuberculosis in adults infected with HIV: systematic review of randomized placebo controlled trials. *Br Med J* 1998; 317: 625-9.

World Health Organization. Global programme on AIDS and tuberculosis programme. *Wkly Epidemiol Rec* 1989; 64: 125-31.

Yanai H, Uthaivoravit W, Panich V, *et al*. Rapid increase in HIV-related tuberculosis, Chiang Rai, Thailand, 1990-1994. *AIDS* 1996, 10: 527-31.