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A Comparative Study of Tuberculin Skin Test Reactivity Between Asymptomatic HIV-1 Seropositive Subjects and Healthy Volunteers

Surapol Suwanagool, Varaporn Chuenarom[1], Ladda Pechthanon[1], Areerat Sonjai, Amorn Leelarasamee[2] and Chaiporn Pathrakom[3]

SUMMARY **During November 1993 - October 1994 tuberculin skin test reactivity (PPD-Thai Red Cross: 0.1 ml of 10 IU) was determined among 399 asymptomatic HIV-1 positive subjects and 405 healthy volunteers, 10% (40/399) had PPD-TRC induration 0-2 mm compared with 4.2% (17/405) ($p = 0.001$) and 43.4% (173/399) had induration ≥ 10 mm compared with 53.8% (218/405) ($p = 0.003$) of healthy volunteers. However, the percentage of the PPD-TRC induration 5-9 mm was similar among HIV-1 seropositive subjects and healthy volunteers as 37.6% (150/399) vs 34.8% (141/405) ($p = 0.4$). The mean PPD-TRC reaction of HIV-seropositive subjects were 6.4 ± 0.9 mm vs. 11.0 ± 0.5 mm among those with CD_4 lymphocyte counts 200-299 cells/mm^3 compared with those ≥ 300 cells/mm^3 ($p < 0.001$). We provide support for use of induration of ≥ 5 mm of PPD-TRC skin reaction for evidence of latent infection with *Mycobacterium tuberculosis* as the CDC recommendation in asymptomatic HIV-seropositive subjects. Consideration of tuberculosis chemoprophylaxis should have benefit, particulary in areas where *M. tuberculosis* is highly prevalent such as Thailand. However, among HIV-1 seropositive carriers with negative tuberculin (PPD-TRC) skin tests, there needs to be a careful evaluation and follow-up for evidence of tuberculous infection.**

The pandemic of human immunodeficiency virus (HIV-1) has had a profound impact on tuberculosis globally.[1,2] WHO has estimated that in 1994 among 14 million HIV-infected people, 5.6 millions were co-infected by both tuberlosis and HIV.[3] Overall, an average 3.8 million cases of tuberculosis were reported annually in the period 1990-1993 and 48.6% of these lived in the South-East Asia region.[4] Most tuberculosis in HIV-positive patients results from reactivation of a previously acquired latent infection.[5]

In order to diagnose tuberculous infection, the tuberculin skin test remains the standard diagnostic method. Measurement of the skin induration within 48-72 hours after intradermal injection of tuberculin or purified protein derivative (PPD) is the simplest, most frequently used technique of infering tuberculous infection.[6] The sensitivity and specificity of this test are affected by several factors, including decreased cellular immunity due to HIV-1 infection. In order to identify latent tuberculous infection in asymptomatic HIV-1 seropositive carriers in our community, we evaluated PPD skin test reactivity in asymptomatic HIV-1 seropositive and seronegative Thai subjects via the Mantoux technique using 0.1 ml of purified protein derivative produced by the Science Division of the Thai Red Cross (PPD-TRC).

MATERIALS AND METHODS

The study participants were voluntarily recruited from asymptomatic HIV-infected subjects attending the Infectious Disease and Counselling Clinic (operated by Department of Preventive and Social Medicine), the Women's Counselling Clinic (operated by the Out-Patient Gynecology Clinic), the

From the Department of Preventive and Social Medicine, [1]Division of Nursing, [2]Department of Medicine, [3]Department of Obstetric Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand,

Correspondence : Surapol Suwanagool, Department of Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Siriraj Clinic for Laborers Going Abroad and from blood donors at Siriraj Hospital. The asymptomatic, HIV-infected subjects comprised 201 males, 198 females ranging in age from 14–48 years (mean age 28.7 ± 0.4 years). None reported a history of intravenous drug use or homosexual behavior. The participants were enrolled in the study if (a) the chest X-ray film showed no pleuro-pulmonary lesion as read by the radiologist who was unaware of the HIV-1 serologic status of the subjects; (b) the CD_4 lymphocyte level, as calculated by flow cytometric procedures, was more than 200 cells/mm^3, and (c) if the subject's condition did not meet the definition for AIDS or symptomatic HIV disease as defined by the Ministry of Public Health, Thailand and Centers for Disease Control, USA, 1993.[7,8] Apparently healthy volunteers in the same range of age groups were recruited from the Siriraj Clinic for Laborers Going Abroad (in which HIV antibody testing and chest X-ray film are required by the employing countries) and 139 assistant-nurse students. The healthy volunteers comprised 202 males, 203 females ranging in age from 17–45 years (mean age 24.2 ± 0.4 years). Purified protein derivative produced by the Science Division of the Thai Red Cross (called PPD-TRC; originally human tuberculin PPD manufactured by CSL, Australia) was used in this study. Using this PPD-TRC it was determined that 0.1 ml or 10 IU were equivalent to standard 0.1 ml or 5 IU of purified protein derivative-S (PPD-S), the Science Division of the Thai Red Cross has previously studied skin test reactivity on 58 adult healthy volunteers comparing PPD-TRC with PPD-S and found that 62% and 64% of the subjects had an induration greater than 10 mm, respectively, with a mean induration of 10.8 mm to PPD-TRC and 12.01 mm to PPD-S; there were no statistically significant differences between reactions of the two products.[9] In this study the test was performed using the Mantoux technique by injection 0.1 ml PPD-TRC intradermally on the volar surface of the forearm by trained nurses. The diameter of induration was measured perpendicular to the long axis of the forearm in mm using calipers, by another two experienced nurses within 48–72 hours after the intradermal injection. The interpretation of the induration (mm), according to the National Tuberculosis Advisory Council (Canberra, Australia) was considered to be negative (<5 mm diameter), weak positive (5–9 mm diameter), positive (10–14 mm diameter) and strong positive (15 mm diameter or more). A questionnaire was administered by the authors and included items on socio-demographic characteristics, history of BCG vaccination, evidence of BCG scar and other pertinent information.

RESULTS

From November 1993 to October 1994, 399 asymptomatic HIV-1 seropositive carriers, and 405 healthy volunteers were enrolled in the study. Approximately 75% of the participants belonged to the low socioeconomic stratum. The vast majority had had primary education, practiced unskilled labor as their occupation and were from the northeastern region of Thailand. The demographic characteristics, history of BCG vaccination and BCG scar data of asymptomatic HIV-1 seropositive subjects and healthy volunteers are shown in Table 1. As examined by the authors, none of the participants had a history, physical symptoms or chest X-ray that would be indicative of tuberculosis (pulmonary, extrapulmonary). Determination of CD_4 lymphocyte count, performed by flow cytometric procedures on

Table 1. Demographic characteristics, BCG vaccination history of asymptomatic HIV-1 seropositives versus healthy volunteers.

Total (n=804)	HIV-1 antibody positive subjects (n=399)	Healthy volunteers (n=405)
Age (mean)	28.7 ±0.4 years	24.2 ±0.4 years
Sex : male	201	202
female	198	203
Marital status:		
single	78	277
married	284	124
widowed	24	2
missing	13	2
History of BCG vaccination:		
BCG scaring	243	358
no BCG scar	137	45
missing	19	2

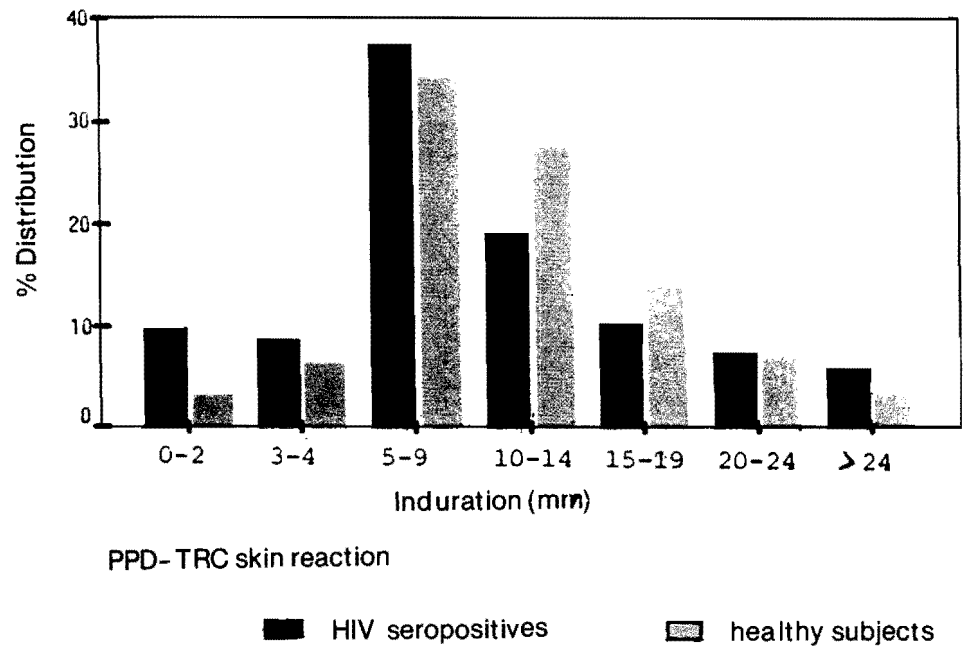

Fig. 1 Percentage distribution of tuberculin (PPD-TRC) skin test reactivity among asymptomatic HIV-1 seropositives and healthy subjects by mm.

Table 2. PPD-TRC skin test reaction of asymptomatic HIV-1 seropositives and healthy volunteers.

Skin test reaction (induration : mm)	HIV-1 seropositives (n=399)	Healthy volunteers (n=405)	p value
0-2	40 (10%)	17 (4.2%)	0.001
3-4	36 (9%)	29 (7.2%)	0.3
5-9	150 (37.6%)	141 (34.8%)	0.4
⩾10	173 (43.4%)	218 (53.8%)	0.003

blood from 399 asymptomatic HIV-1 seropositive subjects, revealed a range of values from 203 to 1,146 cells/mm^3, with a mean 467.4± 158.5 cells/mm^3. We performed tuberculin skin test (PPD-TRC, 10 IU) by intradermal injection 0.1 ml of PPD-TRC in all eligible participants. The measurements of induration (mm) with calipers were interpreted within 48-72 hours after injection. As shown in Fig. 1 and Table 2, of the 399 HIV-seropositive subjects, 40 (10%) had induration 0-2 mm compared with 17 (4.2%) among healthy volunteers (p=0.001). One hundred and fifty HIV-1 seropositive carriers (37.6%) and 141 (34.8%) of healthy volunteers had induration of 5-9 mm, respectively (p=0.4). The mean diameter of induration among HIV-1 seropositive carriers was 10.3±0.4 mm, compared with 11.1±0.3 mm among healthy volunteers (p=0.16) (Table 3). However, induration > 10 mm was present in 173 HIV-1 seropositive carriers (43.4%) and 218 healthy volunteers (53.8%) (p=0.003). Table 3 presents a comparison of PPD-TRC skin test reactions among HIV-1 seropositive subjects related to BCG scar and CD_4 lymphocyte count. The mean PPD-TRC skin test reaction was 6.4±0.9 mm among HIV-1 seropositive carriers with a CD_4 lymphocyte count of 200-299 cells/mm^3 compared with 11.0±0.5 mm among those with CD_4 lymphocyte ⩾ 300 cells/mm^3. Regarding the presence of Bacille Calmette-Guerin (BCG) scar, the mean PPD-TRC skin test reaction was 9.7±0.5 mm compared with 11.6±0.8 mm

Table 3. Comparison of PPD-TRC skin test reaction of asymptomatic HIV-1 seropositives versus healthy volunteers and relation to BCG scar, CD_4 lymphocyte count.

	Number	% Induration >10 mm	Mean PPD-TRC reaction (mm)	p value
Asymptomatic HIV-1 seropositives	399	43.4	10.3±0.4	0.16*
- with BCG scar	243	38.7	9.7±0.5	0.04
- no BCG scar	137	50.4	11.6±0.8	
- CD_4 lymphocyte 200-299 cells/mm^3	43	11.6	6.4±0.9	<0.001
- CD_4 lymphocyte ⩾300 cells/mm^3	340	47.6	11.0±0.5	
Healthy volunteers	405	53.8	11.1±0.3	

*compared mean PPD-TRC of total asymptomatic HIV-1 seropositive versus healthy volunteers

among those without BCG scars (p=0.04). In addition, among the healthy volunteers, it was found that the mean PPD-TRC skin test reaction was 10.7±0.3 mm among the participants with BCG scars (n=358) compared with 13.7±0.9 mm in those without a BCG scars (n=45, p=0.003).

DISCUSSION

Tuberculin skin test reactivity depends on intact cellular immunity. HIV infection characteristically impairs cellular immunity and results in anergy to common antigens including PPD.[10] In previous studies induration of <10 mm after PPD skin testing occurred in >60% of AIDS patients with active tuberculosis[11,12] and 35% of HIV-1 seropositive patients with active tuberculosis.[13-15] However, PPD skin testing remains a useful, simple method for identifying individuals with latent tuberculous infection. WHO and the Centers for Disease Control (CDC), USA have recommended that PPD skin test positivity be regarded as 5 mm or greater of induration in HIV-infected individuals rather than the standard ⩾10 mm.[16,17] In this study, the majority of HIV-I seropositive subjects and healthy volunteers were recruited from the same socioeconomic strata and were similar in age, sex distribution. It was found that 43.4% of HIV-1 seropositive subjects had induration in reaction to PPD-TRC ⩾ 10 mm compared with 53.8% of healthy volunteers (p=0.003) (Table 2), and when considering the number of CD_4 lymphocytes and evidence of BCG vaccination among HIV-1 seropositive subjects it was found that there were statistically significnat differences of mean induration in reaction to PPD-TRC among groups with CD_4 lymphocyte count of 200-299 cells/mm^3 compared with those ⩾ 300 cells/mm^3 (p<0.001), (Table 3). This suggests that the decreased reactivity observed in the HIV-1 seropositive carriers was a result of HIV-1 infection. Regarding BCG vaccination, there were statistically significant differences of mean induration in reaction to PPD-TRC among HIV-1 seropositive carriers in those with and without BCG scar (p=0.04) (Table 3). Nevertheless, we cannot conclude scientifically whether BCG vaccination after an interval of 20-25 years or the prevalence of tuberculous infection in the population tested is the more important determinant of the effect on tuberculin reactivity. However, the percentage of induration 5-9 mm among HIV-1 seropositive carriers was similar to the healthy volunteers (37.5% vs 34.8%) (p=0.4) (Table 2). Assuming HIV-1 infection results in decreased skin test reactivity to PPD-TRC and in view of the observation that coexisting human immunodeficiency virus infection in new tuberculosis patients in Thailand has increased steadily from 3.8% (1991), 5.6% (1992), 7.1% (1993) to 10.0% (mid 1994),[18,19] our study favors the CDC recommendation of induration of ⩾5 mm reaction to PPD-TRC in HIV-1 seropositive individuals as a positive test for *Mycobacterium tuberculosis* infection rather than standard ⩾10 mm induration in normal individuals. In other studies the sensitivity of PPD in HIV-1 infected population using the 5 mm cut-off point was variable, eg., 56% in intravenous drug users,[10] 60% in pregnant women in Uganda[20] and 64.5% in Haiti.[21] Thus, in practice it is important to evaluate tuberculous infection in HIV seropositive individuals with negative tuberculin skin tests more carefully.

There are reports of prospective studies of the risk of developing active tuberculosis among HIV-seropositive with a positive PPD skin test as 7.9 and 6.9% per 100 patient-year, respectively[22,23] and cohort studies of HIV-infected individuals at risk for tuberculosis suggest that INH prophylaxis is effective.[24,25] The CDC in 1989 recommended HIV-seropositive individuals, who have a positive skin test reaction (≥5 mm induration), should receive isoniazid preventive therapy for a minimum of 12 months. With the increased prevalence of HIV and tuberculous infection in Thailand, tuberculosis chemoprophylaxis should be considered in HIV-seropositive individuals with PPD-TRC skin test induration ≥ 5 mm with no evidence of active tuberculosis, in order to decrease the incidence of tuberculosis. Whether or not it would be appropriate to administer chemoprophylaxis for tuberculosis for HIV-infected persons with negative tuberculin skin tests who live in regions where tuberculosis is highly prevalent or among injecting drug users needs to be further determined.

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