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Tuberculin Skin Test Conversion and Reactivity Rates among Adults with and without Human Immunodeficiency Virus in Urban Settings in Ethiopia

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To investigate whether low CD4$^+$ T-cell counts in healthy and human immunodeficiency virus (HIV)-infected Ethiopians influence tuberculosis (TB) immunological memory, tuberculin skin test (TST) conversion and reactivity rates were investigated among adults with and without HIV infection in urban settings in Ethiopia. Reaction to the TST was analyzed with purified protein derivative by the Mantoux technique. A total of 1,286 individuals with TST results of ≥5-mm (n = 851) and ≤4-mm (n = 435) induration diameters were included. Individuals with ≤4-mm induration sizes were followed up for 21.4 ± 9.5 months (mean ± standard deviation) to observe skin test conversion. The overall TST reactivity (≥5-mm induration diameter) was 66.2% (n = 851). Reactivity was significantly lower among HIV-positive persons (40.5%) than among HIV-negative persons (68.7%) ($P < 0.001$). Of the above persons, 32 incident TB patients were checked for their TST status 13.05 ± 11.1 months before diagnosis and reactivity was found among 22 (68.7%) of them. Of the TST-negative persons with 0- to 4-mm indurations who were followed up for 3 years, the conversion rate to positivity was 17.9/100 person-years of observation (PYO) (14.4/100 PYO and 18.3/100 PYO in HIV-positive and -negative persons, respectively). Despite lower absolute CD4$^+$ T-cell numbers in Ethiopians, higher TST conversion and reactivity rates show the presence of a higher rate of latent TB infection and/or transmission. The lower TST positivity rate before a diagnosis of TB disease showed the lower sensitivity of the test. This indicates the need for other sensitive and specific diagnostic and screening methods to detect TB infection, particularly among HIV-positive persons, so that they can be given prophylactic isoniazid therapy.

Tuberculosis (TB) is still one of the major health threats in the world. One-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*. The majority of the affected people are living in developing parts of the world, especially in sub-Saharan Africa. TB accounts for nearly 6% of all deaths worldwide by killing every fourth person of the eight to nine million people who develop the disease (19) and is the foremost cause of death from a single infectious agent in adults (1). Infection with human immunodeficiency virus (HIV) substantially increases the risk of developing clinically apparent TB (29).

The tuberculin skin test (TST) done with purified protein derivative (PPD) by the Mantoux method, which is highly sensitive but less specific than other cell-based methods, is useful for screening for TB infection in vivo (8, 14, 27). It is reported to be less sensitive among immunocompromised individuals such as those infected with HIV (5, 7, 21). Its prevalence of reactivity is reported to be positively correlated with absolute counts of CD4$^+$ and total lymphocytes (22). Despite its poor specificity, TST is still thought to be useful to diagnose TB infection in non-HIV-infected individuals (11, 16).

M. tuberculosis (the causative agent of TB) and HIV are the most common infectious agents in Ethiopia as 50% of the TB-infected individuals are HIV infected (9, 10, 12, 32). Therefore, it is expected that a high proportion of Ethiopians could be exposed to TB and have an immunological memory for TB antigens. In this country, the national bacille Calmette-Guerin (BCG) vaccination coverage at birth was reported to be 67% in 1997 and 72% in 2004 (13). However, few national surveys have been done on the tuberculin response rate in Ethiopia (3, 15). In addition, a lower absolute CD4$^+$ T-cell count among healthy Ethiopian individuals compared to other populations in the world, including African countries, was observed (32, 33). However, whether this lower absolute CD4$^+$ T-cell count among apparently healthy, HIV-negative individuals influences the TST conversion, reactivity, or positivity rate is unknown. Furthermore, to our knowledge, no study was conducted on the TST conversion rate and its relationship to absolute CD4$^+$ T-cell counts in HIV-infected individuals in Ethiopia. Therefore, in this study, we assessed the rates of TST conversion, reactivity, and positivity and their correlation with CD4$^+$ T-cell counts among HIV-infected and non-HIV-infected adult Ethiopians enrolled in a longitudinal cohort study using the TST.

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MATERIALS AND METHODS

Study subjects. To investigate TST reactivity and the rate of conversion to positivity among adult Ethiopians, we analyzed longitudinal data from the HIV/AIDS natural history cohort study of ENARP at the Ethiopian Health and

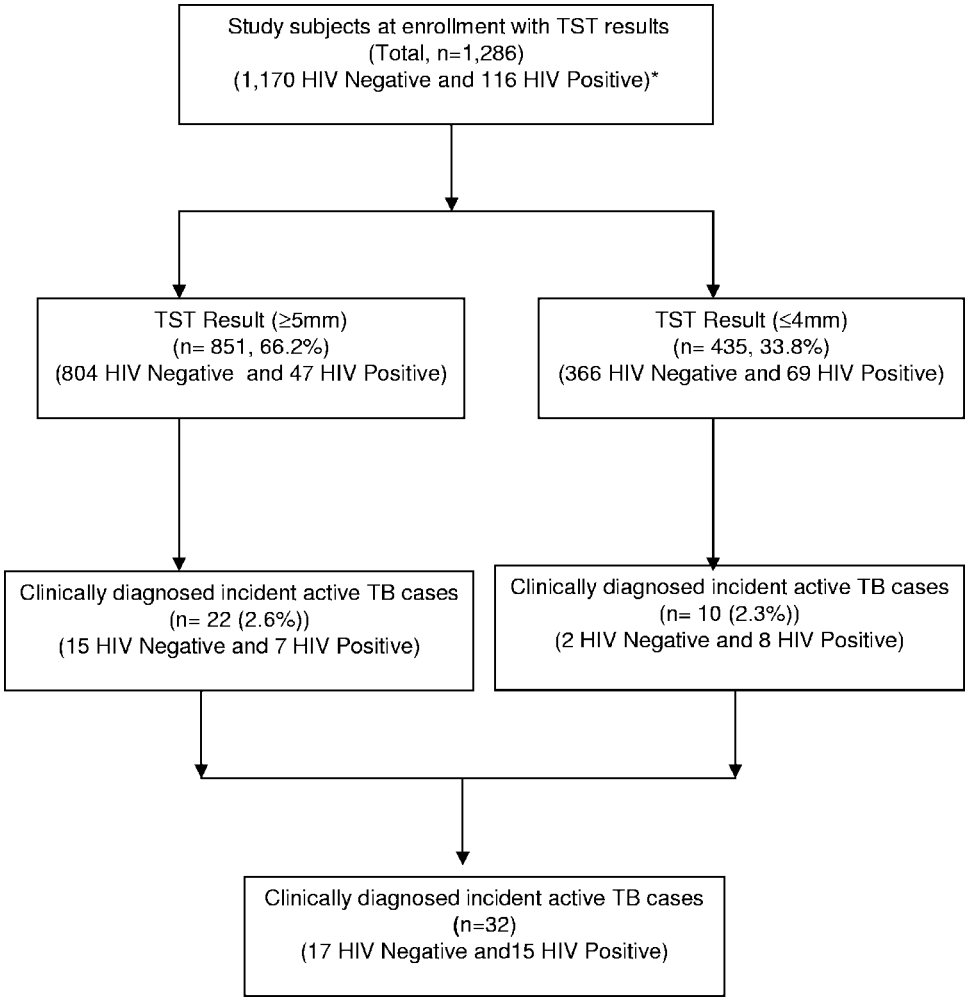

FIG. 1. Flowchart for the selection and composition of the subjects of this study. (*) This was a longitudinal HIV/AIDS natural history study conducted by ENARP at the Ethiopian Health and Nutrition Research Institute from 1995 to 2003 in Addis Ababa, Ethiopia.

Nutrition Research Institute, Addis Ababa, Ethiopia. Of 1,701 enrolled cohort participants (since 1995), a total of 1,286 individuals with complete TST results at enrollment were included in this study. The study subjects were grouped into three categories, (i) individuals TST reactive (≥5-mm-diameter skin test result, $n = 851$) at enrollment, (ii) those with TST results of ≤4-mm induration diameter ($n = 435$) at enrollment and followed up from 1997 to 2000 for 21.4 ± 9.3 months (mean ± standard deviation [SD]) to observe TST conversion, and (iii) those with clinically diagnosed incident TB cases ($n = 32$). The third group includes those who were not TB patients at enrollment but were diagnosed as TB patients who developed the disease in the follow-up period (Fig. 1). Each participant in the cohort was followed up every 6 months at the clinics of the cohort sites by a full clinical examination. Study participants were factory workers who were living in urban settings, a suburb of Addis Ababa (25 km from the capital city), and 114 km southeast of Addis Ababa. The details of the study sites and study conditions have been described elsewhere (30). All HIV-infected patients were antiretroviral agent naive. Characteristics and selection of the study subjects are depicted in Table 1 and Fig. 1.

TST. The TST was done by the Mantoux method. At the forearm, 0.1 ml of PPD (RT-23 SSI 2TE; Statens Serum Institute, Copenhagen, Denmark) was intradermally injected and results (induration diameters) were read by an experienced physician after 48 to 72 h. Two perpendicular diameters of the skin induration were measured, and the average was taken for interpretation. The interpretation was based on four cutoff values: anergy or no response (0-mm induration diameter), negativity (>0- to ≤4-mm induration diameter), reactivity (≥5-mm induration diameter), and positivity (≥10-mm induration diameter). However, TST results for HIV-positive persons were considered positive if induration sizes were ≥5 mm according to the recommendation given by the National Tuberculosis and Leprosy Control and Prevention Program, Ministry of Health, Ethiopia (7, 10). TST conversion to positivity was indicated by an increase in induration diameter of 10 mm or more over a previously negative TST result (<5-mm induration diameter) over a period of 2 years (7, 20, 23, 28). Conversion rates are expressed in person-years of observation (PYO).

Laboratory methods. (i) HIV testing and T-cell subset determination. Blood samples collected from each participant during each visit were tested for HIV with HIVSPOT (Genelab Diagnostics, Singapore) and/or Determine (Abbott Laboratories) and enzyme-linked immunosorbent assay (Vironostika Uniform II PLUS O; Organon Teknika, Boxtel, The Netherlands). Western blotting (HIV Blot 2.2; Genelab Diagnostics, Singapore) was used to confirm reactive samples. Lymphocyte subsets were analyzed by standard three-color flow cytometry (FACScan; Becton Dickinson, San Jose, CA). Plasma HIV RNA levels were analyzed by a nucleic acid sequence-based amplification assay (Organon Teknika, Boxtel, The Netherlands).

(ii) Diagnosis of *M. tuberculosis* infection. Diagnosis of *M. tuberculosis* infection was done by sputum acid-fast bacillus staining for 3 consecutive days for each suspected patient. A patient was considered positive, at least, if two acid-fast bacillus-stained sputum samples positive for the bacilli were identified by smear microscopy. Furthermore, culture was performed if requested by the physicians. The diagnosis of TB was also supported by chest X-ray and pathology. Treatment was provided according to the guidelines of the Ministry of Health, Ethiopia (10).

Ethical consideration. This study is a part of a longitudinal study of the natural history of HIV/AIDS in Ethiopia which was ethically cleared by the National

TABLE 1. Characteristics of the total study population, those with TST results of ≥5 mm (reactive cases) and ≤4 mm (follow-up cases), and those with active TB incident cases

Characteristic	Total (n = 1,286)	TST result of ≥5 mm (n = 851)	TST result of ≤4 mm (n = 435)	Incident TB cases (n = 32)
No. (%) of males	998 (78.1)	665 (78.1)	333 (76.5)	26 (81.2)
Age [yr (IQR)][a]				
HIV-positive persons	33 (30–39)	32 (30–39)	34 (30–39)	35 (30–38)
HIV-negative persons	35 (29–40)	35 (29–40)	35 (29–40)	39 (32–42)
No. (%) of persons with BCG scar	535 (41.6)	385 (45.2)	150 (34.5)	9 (28.1)
No. (%) of persons with HIV antibodies	116 (9.0)	47 (5.5)	69 (15.9)	15 (46.9)
No. of $CD4^+$ T cells/μl (IQR)				
HIV-positive persons	326 (197–483)[b]	363 (252–483)[b]	306 (167–462)[b]	237 (140–375)
HIV-negative persons	693 (544–853)	713 (576–865)	660 (509–819)	NA[c]
Total no. (%) of persons with $CD4^+$ T-cell level (cells/μl) of:				
<200	29 (6.2)	7 (24.1)[d]	22 (11.8)	NA
≥200	441 (93.8)	277 (62.8)	164 (88.2)	
Log_{10} no. of plasma HIV RNA copies/ml (IQR)	3.9 (3.2–4.7)	3.9 (3.3–4.6)	3.8 (3.3–4.8)	4.3 (3.4–4.9)

[a] IQR, 25th to 75th quartiles.
[b] $P < 0.001$ compared to HIV-negative persons by Mann-Whitney U test.
[c] NA, complete data not available.
[d] $P < 0.001$ compared to ≥200-cell/μl $CD4^+$ T-cell count (χ^2 test).

Ethical Committee. Each study participant gave consent to participate in the study.

Statistical analysis. Analysis was performed with STATA (Intercooled STATA, version 7; Stata Corporation, College Station, TX). TST reactivity differences (medians) were compared by the nonparametric Mann-Whitney U test. A P value of less than 0.05 was considered indicative of statistical significance. The incidence of TST conversion was computed by assuming a Poisson distribution of events. Kaplan-Meier analysis was performed to compare conversion rates by HIV status. Incidence curves were compared by a log-rank test.

RESULTS

Characteristics of study subjects. A total of 1,286 study subjects had complete TST results. The percentage of males was higher (77.6%). Age was not significantly different between males (34 years; interquartile range [IQR], 29 to 40 years) and females (35 years; IQR, 30 to 39 years; $P = 0.284$) or between those with and those without HIV ($P = 0.78$). Of the total study subjects, 116 (9.0%) were HIV positive. HIV-negative persons had higher absolute $CD4^+$ T-cell counts (693 cells/μl; IQR, 544 to 853) than HIV-positive persons (326 cells/μl; IQR, 197 to 483; $P < 0.001$) (Table 1).

TST reactivity. In 1,286 participants with TST results at enrollment, the overall reactivity rate (≥5-mm induration diameter) was 851 (66.2%) and was not different by year (1997 to 1999). The reactivity rate of HIV-negative persons (68.7%) was higher than that of HIV-positive persons (40.5%) ($P < 0.001$). TST positivity (≥10-mm induration diameter) among HIV-negative persons was 56.1% (Fig. 2). Skin test induration sizes were higher in HIV-negative persons (median, 10 mm) than in HIV-positive persons (median, 4 mm; $P < 0.001$) (data not shown). However, the presence of anergy (0-mm induration diameter or no response) was higher (45.7%) in HIV-positive persons than in HIV-negative persons (24.4%; $P < 0.001$) (Fig. 2). Of 1,284 persons with BCG results, 535 (41.7%) had a scar.

TST reactivity was higher in those individuals with a BCG scar than in those without a scar (71.9% versus 62.1%, $P < 0.001$), which might be the effect of BCG. Furthermore, among those without a BCG scar, reactivity was higher in HIV-negative persons (64.1%) than in HIV-positive persons (43.1%; $P < 0.001$) (data not shown).

TST response in relation to the absolute $CD4^+$ T-cell count. To investigate whether TST reactivity is influenced by the degree of HIV infection (14), we analyzed $CD4^+$ T-cell numbers in relation to reactivity. In TST-reactive cases, HIV-negative persons had higher absolute $CD4^+$ T-cell counts (713 cells/μl; IQR, 576 to 865 cells/μl) than HIV-positive persons (363 cells/μl, 252 to 483 cells/μl) ($P < 0.001$). TST-reactive persons (≥5-mm induration diameter) had higher absolute $CD4^+$ T-cell counts (673.5 cells/μl; IQR, 511 to 822.5 cells/μl) than anergic (no response) persons (538 cells/μl; IQR, 333 to 761; data not shown) ($P < 0.001$). When the absolute $CD4^+$ T-cell counts were classified into two categories (<200 and ≥200 cells/μl), TST reactivity was higher in those with absolute $CD4^+$ counts of ≥200 cells/μl (24.1% versus 62.8%, $P < 0.001$), whereas anergy (no response) was higher in those with <200 cells/μl (72.4% versus 30.8%, $P < 0.001$) (Table 1). The same was true of HIV-positive individuals. Logistic regression analysis showed an increase in TST reactivity with a BCG scar (odds ratio [OR], 1.5; confidence interval [CI], 1.1 to 1.9; $P < 0.01$) and a decrease with HIV infection (OR, 0.39; CI, 0.26 to 0.57; $P < 0.001$). Reactivity increased with the absolute $CD4^+$ T-cell number in the study subjects (OR, 1.00; CI, 1.00 to 1.003; $P < 0.001$).

TST conversion rate. To analyze the TST conversion rate, 435 individuals (0- to 4-mm induration diameters at enrollment) were followed up for a mean follow-up time of 21.4 months (±9.3 months [SD]). With an induration diameter in-

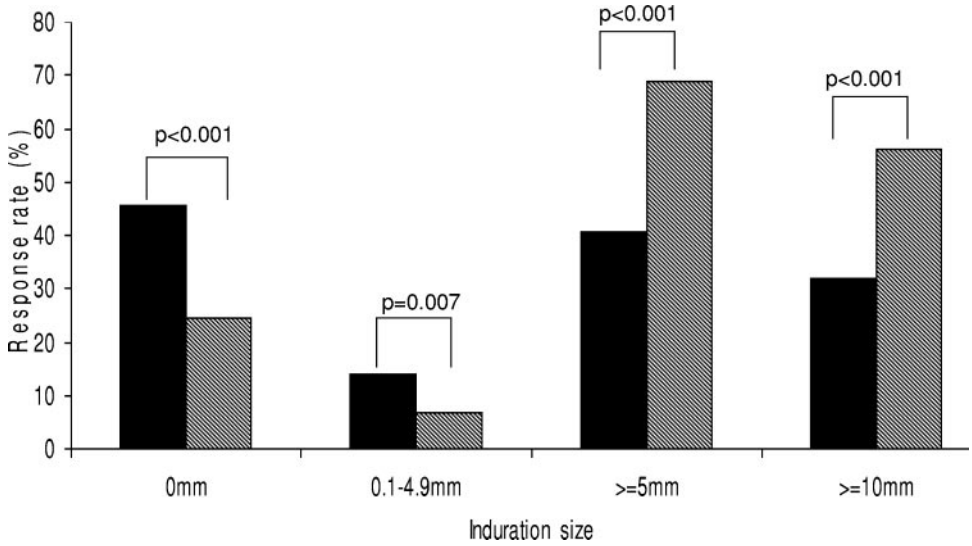

FIG. 2. TST response rates among all of the persons (n = 1,286) included in this study at different TST induration sizes for HIV-negative (gray bars) and HIV-positive (black bars) individuals (including the incident TB cases in both groups).

crease of ≥10 mm from the previous negative result as the cutoff, the conversion rate was 14.3/100 PYO (CI, 7.9 to 25.9/100 PYO) and 18.3/100 PYO (14.8 to 22.6/100 PYO) in HIV-positive and -negative persons, respectively (P = 0.453). Even after using an induration diameter cutoff value for conversion in HIV-positive persons of ≥5 mm (since reactivity for HIV-positive persons was defined as an induration diameter of ≥5 mm), the conversion rate was 16.2/100 PYO (CI, 9.2 to 28.6/100 PYO), which was not statistically significantly different from the ≥10-mm induration diameter increase. Among HIV-negative persons, an increasing trend in TST conversion was observed in the years 1997 (14.9/100 PYO), 1998 (21.5/100 PYO), and 1999 (20.6/100 PYO). Moreover, a higher rate of conversion (30.2/100 PYO; CI, 22.3 to 40.9/100 PYO) was

TABLE 2. TST conversion rates among 435 persons with ≤4-mm indurations followed up from 1997 to 2000

Parameter	Conversion rate			
	No. of conversions/ no. of PYO	Per 100 PYO	95% CI	P value[a]
Overall TST conversion (≥10-mm increase)[b]	99/552.6	17.9	14.7–21.8	
HIV-positive persons with ≥5-mm increase	12/73.9	16.2	9.2–28.6	
HIV-positive persons with ≥10-mm increase	11/76.4	14.4	7.9–25.9	
HIV-negative persons with ≥10-mm increase	85/464.6	18.3	14.8–22.6	NS[c]
BCG scar (HIV-negative persons):				
Yes	42/139.1	30.2	22.3–40.8	
No	43/325.6	13.2	9.8–17.8	0.0001
Gender (HIV-negative persons)				
Female	11/87.1	12.6	6.9–22.7	
Male	53/230.2	23.0	17.6–30.1	0.06
Yr (HIV-negative persons)				
1997	32/214.3	14.9	10.5–21.1	
1998	33/153.4	21.5	15.2–30.2	
1999	20/96.9	20.6	13.3–31.9	NS
HIV-positive persons with $CD4^+$ cell counts/mm^3 of:				
<200	1/20.4	4.9	0.7–34.7	
≥200	10/50.1	19.9	10.7–37.1	NS

[a] P values were determined by the Mantel-Haenszel method.
[b] The overall rate of conversion was calculated on the basis of an increase of ≥10 mm in skin test induration size since 88.5% of the converters were HIV-negative persons.
[c] NS, no statistically significant difference.

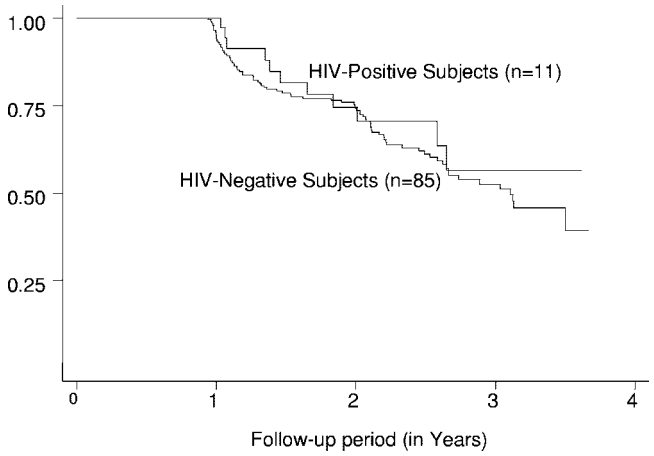

FIG. 3. Kaplan-Meier survival estimates for the rate of TST conversion by HIV status. The *y* axis shows the percent conversion rate.

observed among those HIV-negative persons with a BCG scar compared to those without a scar (13.2/100 PYO; CI, 9.8 to 17.8/100 PYO; $P = 0.0001$) (Table 2). HIV-positive persons with absolute $CD4^+$ T-cell counts of <200 cells/μl had a lower TST conversion rate (4.9/100 PYO; CI, 0.7 to 34.7/100 PYO) than those with absolute $CD4^+$ T-cell counts of ≥200 cells/μl (19.9/100 PYO; CI, 10.7 to 37.1/100 PYO). Almost 50% of the study subjects converted within 2.5 years, and this was not statistically significantly different between HIV-positive and HIV-negative persons (Fig. 3).

TST results among clinically diagnosed incident TB cases. As indicated in Materials and Methods, in this group, 32 incident TB cases (22 with ≥5-mm and 10 with ≤4-mm induration sizes at enrollment) were included to examine TST reactivity and positivity; 17 of them were HIV negative, and 15 were HIV positive. As shown in Fig. 1, the occurrence of TB in the total study group ($n = 1{,}286$) was higher in HIV-positive persons (12.9%) than in HIV-negative persons (1.4%), as reported earlier (32). Among individuals with HIV infection, the median absolute $CD4^+$ cell number and viral RNA level were 237 cells/μl and 4.3 $\log_{10}$ copies/ml of plasma, respectively (Table 1).

On average, at 13.05 ± 11.1 months (mean ± SD) before the diagnosis of clinical TB, 68.7% of the 32 persons had reactive (≥5-mm induration diameter) TST results. Reactivity was higher in HIV-negative persons (88.2%) than in HIV-positive persons (46.7%) ($P < 0.001$). However, a negative response (anergy or a 0-mm induration diameter) was higher among HIV-positive persons (46.7%) than among HIV-negative persons (5.9%) ($P = 0.008$), which clearly indicates the effect of HIV on the test (data not shown).

A second TST result from 21 (65.6%) TB incident cases at an average of 14.7 ± 10.7 months (mean ± SD) after the diagnosis and treatment of TB showed that TST reactivity (≥5-mm induration diameter) was increased to 17/21 (80.9%).

DISCUSSION

TST reactivity and conversion are considered to be indicative of infection of individuals with *M. tuberculosis*. In addition, a higher risk of developing active TB within 2 years following conversion is well documented (2, 4, 20). In this study, we investigated the rates of TST conversion and reactivity in adults with and without HIV infection in urban settings. A TST conversion rate higher than in other reports (4, 26, 28) was found, and it was comparable to the observations in Mexico (24). The higher conversion and reactivity rates among HIV-negative persons despite lower absolute $CD4^+$ T-cell numbers might be attributable to latent TB infection and a higher incidence of TB or transmission of TB in the population (10, 12).

Despite the effect of HIV on TST conversion, reactivity, and anergy (7, 18), the observed reactivity among HIV-positive persons (40.5%) was still lower than those in the United States (52.3%), Haiti (65%), and Uganda (73.8%) (17, 18, 24). This could partially be explained by the presence of lower absolute $CD4^+$ T-cell counts in Ethiopians (33). However, the involvement of other factors such as nutrition and concomitant infection among HIV-positive persons (parasitic infections) could not be ruled out.

The finding of higher absolute $CD4^+$ T-cell counts in TST-reactive persons than in anergic persons is in agreement with other studies (16) and could be due to an increase in the TST response rate when the immune system is intact (22). An increase in the TST reactivity rate among those clinically diagnosed incident TB cases after TB treatment was found. This suggests that TB affects the immune system negatively and that treatment reverses this. Other studies also showed that the in vitro PPD response rate was increased after treatment of helminthic infections among Ethiopians (6).

The lower ability of TST to detect clinically diagnosed TB cases before their clinical presentation of TB, the presence of a higher incidence and prevalence of TB and HIV coinfection in the country (9, 12), and the presence of a higher prevalence of anergy among HIV-positive persons compared to other reports (17, 22) show the need for other sensitive and specific methods to help in the detection of *M. tuberculosis* infection among HIV-infected individuals. This is due to the increased risk that anergic persons will develop TB (16, 25, 31).

The occurrence of equal percentages of TST conversion among HIV-positive and -negative individuals was most probably due to the presence of a higher TB incidence, which might indicate that both groups had equal chances of *M. tuberculosis* exposure and/or infection. However, HIV-positive individuals are at a higher risk of developing TB than HIV-negative persons (32). The occurrence of a higher conversion rate among those individuals with a BCG scar might indicate true conversion due to recent infection than a boosting effect of BCG since these individuals were TST negative at enrollment.

In conclusion, in this study, with the TST as a readout, the higher percentage of reactivity and conversion is probably a reflection of a higher rate of latent TB infection and transmission in the country. However, the lower sensitivity of TST among the incident TB cases, the negative effect of HIV on the test, the absence of sufficient numbers of supportive TB culture facilities, and the lower ability of the smear microscopy diagnostic method (~20% to 50%) to detect TB infection will all have a negative effect on the treatment of persons with undiagnosed cases in a population (who are potential transmitters of TB). Thus, the data presented here will contribute to the efforts made to control and prevent TB infection by increasing the awareness of latent TB infection, conversion rates, and the

problems related to the TST, particularly among HIV-positive persons. Therefore, introducing alternative sensitive, specific, and affordable diagnostic and screening methods for TB into HIV testing schemes could probably help to reduce the spread of TB by allowing cases to be treated earlier and latently infected HIV-positive individuals to benefit from isoniazid prophylaxis.

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