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ORIGINAL ARTICLE

LATENT TUBERCULOSIS AMONG ADULT ETHIOPIAN PATIENTS AT CHEST CLINIC, TIKURANBESSA SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA

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ABSTRACT

Background: *One-third of the world population is infected with Mycobacterium tuberculosis. Most people exposed to M. tuberculosis showed no evidence of active disease. About 5-10% of people with latent tuberculosis infection (LTBI) without HIV will progress to develop active tuberculosis (TB) in their lifetimes. This study was conducted to determine the magnitude of latent TB among the adult population at a teaching and referral Hospital in Ethiopia.*

Methods: *This study was conducted at the Chest clinic of Tikur Anbessa Specialized Hospital during 2010-2013. The study was a cross-sectional study conducted among healthy adults after informed consent was obtained from each individual. Tuberculin skin test (TST) and Interferon Gamma whole blood assay (Quantiferon-Tuberculosis-Gold) was performed using enzyme linked immuno-sorbent assay. Average CD4, CD8, CD3 and CD4:CD8 ratio was determined for all study participants.*

Results: *From a total of 70 healthy adults tested for LTBI using Quantiferon Gold, 45(64%) tested positive and 25 (36%) were negative for latent tuberculosis infection. From the 66 healthy individuals who were tested using TST for LTBI, 42 (62%) individuals were TST positive and 25 (38%) individuals were TST negative. Average CD4, CD8, CD3 and CD4:CD8 ratio was 748, 598, 1401 and 1.4, respectively.*

Conclusions*: The magnitude of latent tuberculosis infection was high in this study, which reflects existing high prevalence of TB. TST and Quantiferon-Tuberculosis-Gold assay show similar efficacy for the diagnosis of LTBI in healthy Ethiopian adults. The absolute CD4 T-cell counts of healthy HIV- negative Ethiopians are considerably lower than CD4 T cell counts in other countries.*

Key Words: *Latent tuberculosis, Chest clinic, adult population, TAH*

INTRODUCTION

One-third of the world population is infected with *Mycobacterium tuberculosis* (1,2). About 5-10% of latent tuberculosis infection (LTBI) without HIV progress to develop active tuberculosis (TB) in their life time. Identification of individuals with LTBI will increase case detection rates and may dictate new treatment policy for the control of tuberculosis (3). Infection with *M. tuberculosis* can result in active TB or, more commonly, latent infection (4). LTBI is defined as an asymptomatic state in people who are infected with *M. tuberculosis* and have no clinical or radiographic evidence of active disease. *M. tuberculosis* resides in the host in a clinically inactive or latent state; contained by the host's immune response. LTBI is currently detected by a standardized, intradermal skin test, called the tuberculin skin test (TST) or by a blood test - QuantiFERON-Gold Test

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(QFT) or Gold-In-Tube, or T-Spot TB in people with risk factors for exposure to mycobacteria and *M. tuberculosis*, respectively (5). Accurate determination of the prevalence of latent infection is essential for an improved understanding of the epidemiology of tuberculosis and for the design and evaluation of tuberculosis control strategies (6).

Estimates of the prevalence of latent *M. tuberculosis* infection have historically been based on TST which has its own limitations (2-4, 7-9). Intradermal inoculation of purified protein derivative (PPD), a crude precipitate of *M. tuberculosis* culture supernatant that contains 1,200 antigens widely shared among mycobacteria other than *M. tuberculosis*, including *M. bovis*, Bacille Calmette-Gu'erin (BCG) and many environmental mycobacteria, elicit a local cutaneous delayed-type hypersensitivity response in sensitized individuals. Currently, advances have provided a new generation of immunoassays, including QFT based on the detection of IFN-γ section by peripheral T cells upon incubation with the specific *M. tuberculosis* antigens ESAT-6 and CFP-10 (10, 11). The ESAT-6 and CFP-10 proteins, secreted by *M. tuberculosis* cells, are potent T-cell antigens which have a role in TB pathogenesis. These antigens are considered to be powerful reagents for the precise detection of LTBI in both vaccinated and unvaccinated populations (11-13).This study aimed to determine LTBI point prevalence in an Ethiopian population using TST and the IFN-γ release assay.

MATERIALS AND METHODS

This was a cross-sectional study of the magnitude of latent TB among a healthy adult population. Following informed consent, 70 healthy adults were prospectively enrolled. TST and Interferon Gamma whole blood assay was performed using ELISA. The study was conducted at the Chest clinic of Tikur Anbessa Specialized Hospital (TASH) during the years of 2010 to 2013. Chest X-ray (CXR) screening was done to ensure that the study participants had no evidence of an asymptomatic TB focus or any TB residual scar from a previous infection.

The study was approved by the Ethics and Scientific Committee of the College of Health Sciences, Addis Ababa University. Written informed consent was obtained from all participating individuals. TST was performed on all participants by administering 0.1 ml containing 5 tuberculin units. Transverse induration was measured 72 hours later using the ballpoint pen technique. A reaction equal to or more than 10 mm was considered positive (14). Trained nurses performed and interpreted the results 72 hours after application according to the American Thoracic Society (ATS) and US Centres for Disease Control and Prevention (CDC) guidelines (15).

For QFT, venous blood of 70 individuals was collected in heparinized tubes provided by the manufacturer of the test kit. Blood was transported to the lab in the upright position with the use of portable blood incubator. The QFT assay was performed in accordance with the manufacturer's instructions. The testing was conducted in two parts, an overnight culture of blood with stimulation by antigens and the subsequent quantification of IFN-γ production. Following an overnight incubation at 37°C in a humidified atmosphere, the supernatant plasma was harvested and then stored at -80°C until the analysis. All samples were assayed for IFN-γ in a single ELISA run. Results were calculated and interpreted according to the manufacturer's instructions. Calculations were performed using software provided by the manufacturer's kit (Analysis Software version 1.51 Cellestis).

The interpretation of IGRAs is based on the amount of INF-γ released, in QFT, or on the number of cells that release INF-γ, in T-SPOT® *TB test.* Laboratories provided both the qualitative and quantitative results. Qualitative results were reported as positive, negative, indeterminate or borderline. Quantitative results were reported as numerical values that included a response to the TB antigen and 2 controls, nil and mitogen. Quantitative results were used for clinical decision making in individual cases, in combination with risk factors (16). In this study we also determined the mean values of CD4, CD3, CD8 and CD4:CD8 ratio for all participants.

The data was analysed using Statistical Software for Social Sciences (SPSS) version 20 and Microsoft (MS) Excel. Descriptive statistics were used to summarize the data, i.e. mean and standard deviation for continuous variables and frequency along with percentages for categorical variables. The levels of IFN-γ cytokine and TST induration for study participants were compared using Student t-test and Chi-square. Pearson correlation test was used to compare TST and IFN-γ. $P<0.05$ was considered significant (17, 18).

RESULT

A total of 70 healthy adult volunteers were enrolled in the study with female to male proportion of 40% to 60%. The age range was from 19 to 75 years and

the mean age was 44. TST and QFT were performed on all patients but four patients did not come back for PPD reading. Those who were assessed with PPD test were only 66 subjects. (Table 1 and Figure 1)

Among a total of 70 healthy adults tested for LTBI using QFT, 45 (64%) tested positive and 25(36%) of them tested negative for latent TB infection. Sixty six healthy individuals were tested using tuberculin skin test for LTBI and results showed that 42 (62%) were positive and 25(38%) were negative. The average age was 28 years for healthy non-TB and 32 years for healthy LTBI positive individuals based on Quantiferon Gold, 26 years for healthy non-latent TB and 30 years for healthy latent TB positive individuals identified using PPD test (Figure 2).

The average CD4, CD8, CD3 and CD4:CD8 ratio of the study participants were 748, 598, 1401 and 1.4, respectively (Figure 3).

Subgroup analysis was made based on their PPD and Quantiferon Gold status as LTBI and no LTBI. For each subgroup, the average CD4, CD8, CD3 and CD4:CD8 ratio in healthy (PPD and Quantiferon gold test negative) individuals were 789, 624, 1472, and 1.4, respectively (Figure 5), which is slightly higher than in the healthy latent-TB positive individuals (Figure 6).

Table 1. Socio-demographic characteristics of 70 adult volunteers screened for latent tuberculosis, Tukur Anbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

Characteristics	Latent TB-Infection (LTBI)		Total (%)
	Positive (%)	Negative (%)	
All participants	45(64%)	25(36%)	70(100%)
Age (quartiles)	32	28	
CD 3 count (quartiles)	1380	1472	1401
CD 4 count (quartiles)	745	789	748
CD8 count (quartiles)	586	624	598
CD4 / CD8 ratio (quartiles)	1.4	1.4	1.4
CXR finding in all	normal	normal	normal

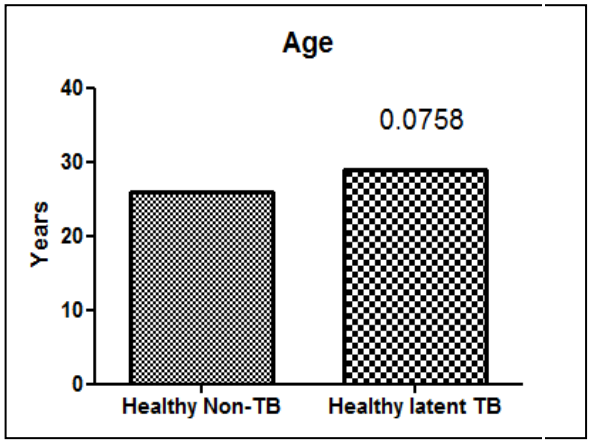

Figure 1. Age distribution of 70 adult volunteers screened for latent tuberculosis by Quantiferon Gold result and PPD status, Tukur Anbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

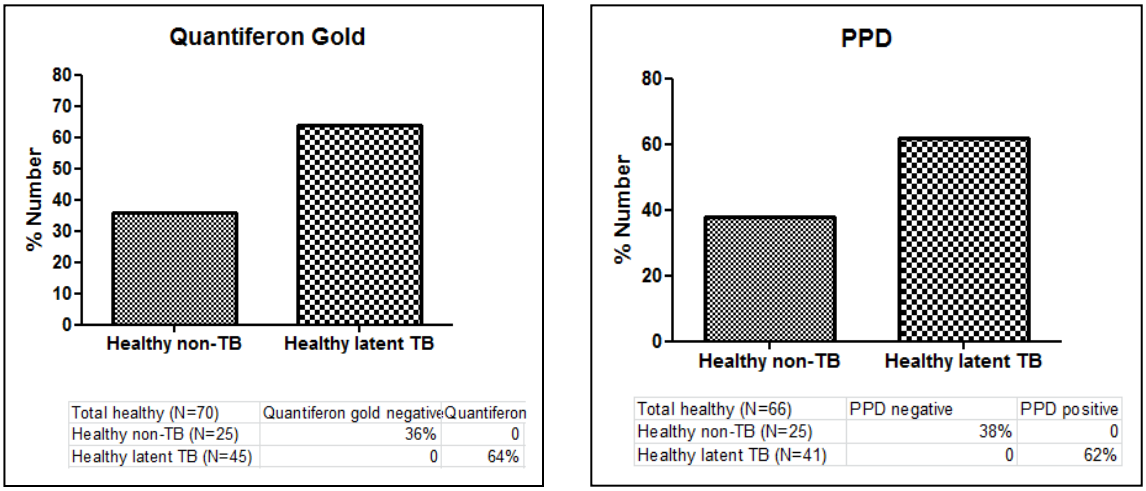

Figure 2: Distribution based on the Quantiferon Gold result and PPD status among 70 adult volunteers screened for latent tuberculosis, Tukur Anbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

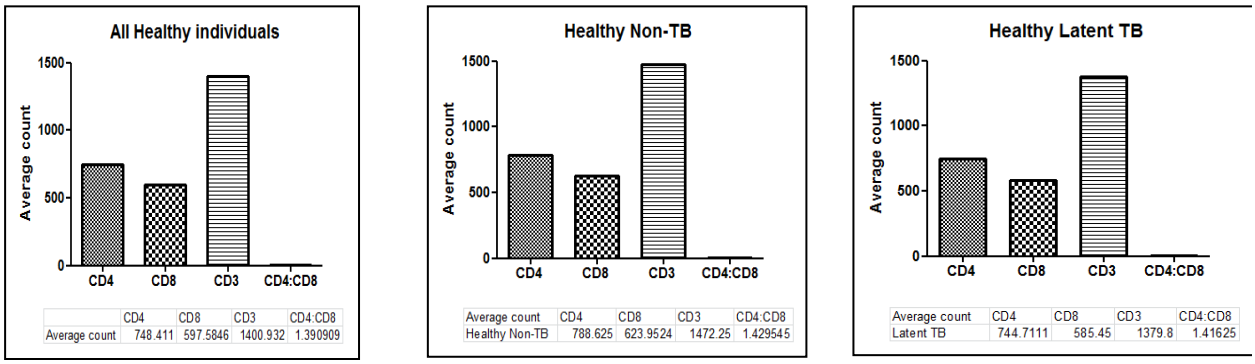

Figure 3: Average CD4, CD8, CD3 and CD4:CD8 ratio in 70 adult volunteers with and without latent tuberculosis, TukurAnbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

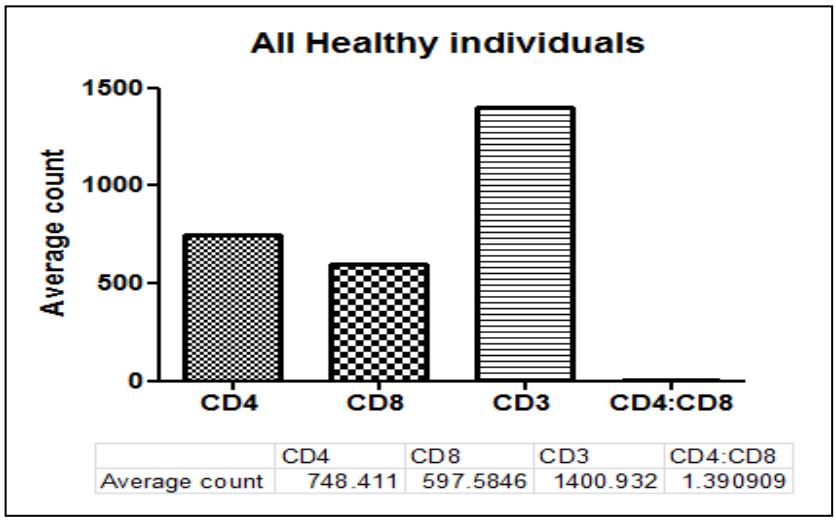

Figure 4: Average CD4, CD8, CD3 and CD4:CD8 ration in Health Adult volunteers, Tukur Anbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

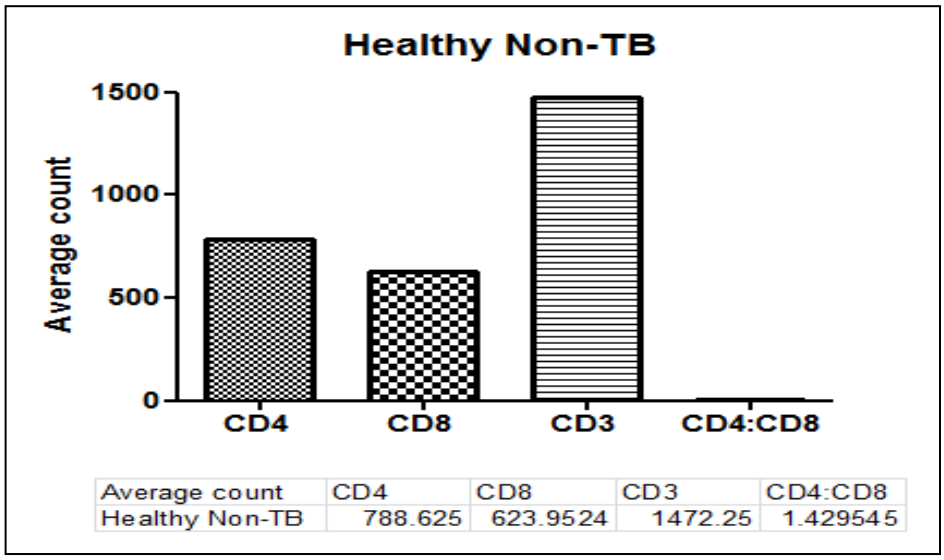

Figure 5: Average CD4, CD8, CD3 and CD4:CD8 in Healthy Non-tuberculosis Adults,Tukur Anbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

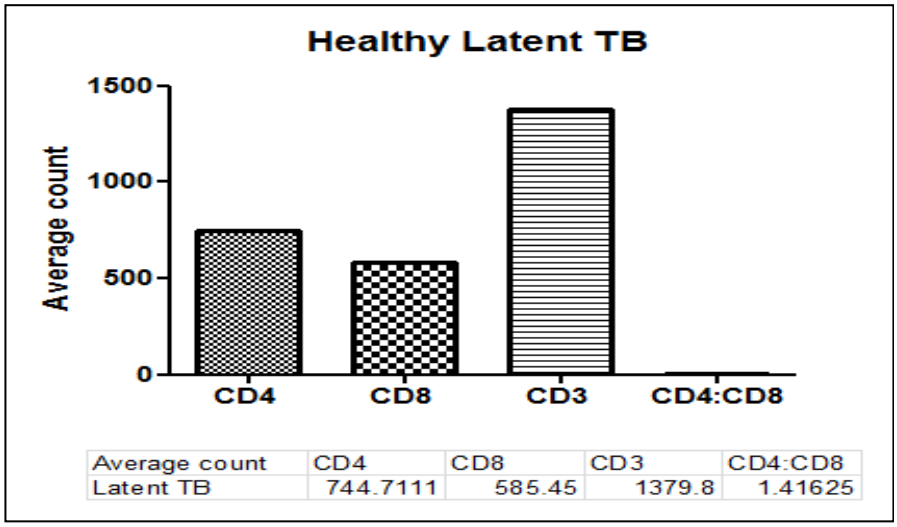

Figure 6. Average CD4, CD8, CD3 and CD4:CD8 in adults with latent tuberculosis**,** Tikur Anbessa Specialized Hospital, 2010-2013, Addis Ababa, Ethiopia

DISCUSSION

Tuberculosis control programs in developing countries are greatly affected by low case detection rates and LTBI infections, which constitute hidden pools that continuously feed into new cases of active TB. As emphasized by some of the previous reports, accurate diagnosis of LTBI and appropriate treatment of cases would reduce the risk of progression to overt disease (19, 20).

Testing of adult TB contacts is controversial in high incidence countries (21, 22). At present, there is no gold standard test for the detection of individuals with LTBI. TST has been the most widely used diagnostic tool for LTBI, now in use for more than a century. TST specificity is reduced in individuals who had BCG vaccination (23). On the other hand, whole blood IFN-γ release assays are highly specific for latent *M. tuberculosis* infection, but are expensive, need special equipment as well as expertise and are not widely available in developing countries. In addition, these tests have limited predictive values (23, 24).

In the absence of a gold standard test for LTBI, we took point prevalence as an objective measure of LTBI. In our study, IFN-γ release assay results were similar to TST in detecting LTBI. These data are in contrast with previous studies that reported that the IFN-γ release assay is better than TST in detecting individuals with LTBI (25-34).

Diagnosis of latent TB in healthy individuals in this country involving a total of 107 participants showed that 46.7% had a positive TST result (TST≥10 mm), 43.9% had a positive QFT-GIT assay result and 44.9% had BCG scar. There was strong agreement between TST (TST ≥10mm) and QFT-GIT assay (Kappa = 0.83, p value = 0.000). But the result was lower than our finding which may show the distribution of LTBI may vary based on the area and demographic factors (17).

In a community-based cross-sectional survey of LTBI using QFT GIT and involving 652 apparently healthy adult pastoralist communities of the Afar Region of Ethiopia it was reported that LTBI prevalence was 63.7%. Even though the setup is not the same, the finding is similar to ours (34).

A study done in north east Ethiopia which assessed the association between the level of IFN-g and tuberculin skin test (TST), showed that there is a strong positive correlation between the level of IFN-γ induced by the specific antigens and the diameter of the skin indurations (Spearman's rho 5 0.6, P < 0.001). Among the 505 subjects who had both TST and QFT GIT test results, 168 (33.3%) were positive with TST (>10 mm), while 326 (64.6%) were positive in QFTGIT (≥0.35 IU ml^{-1} IFN-γ) (35). Our finding of high positivity with QFT GIT is consistent with this report form northern Ethiopia.

Ethiopians had significantly lower mean absolute CD4 T-cell counts (775 versus 993), CD4/CD8 T-cell ratios (1.2 versus 2.2), and B-cell counts (191 versus 313). The opposite was true for CD8 T cells (747 versus 506). However, Ethiopian CD4 T-cell values and CD4/CD8 T-cell ratios were comparable to those reported for Chinese adults (16), which confirms and extends previous reports of low CD4 T-cell counts in Ethiopians (17,18). High prevalence of infections and nutritional factors has been indicated as possible contributors to the reduced CD4/CD8 T-cell ratios (18). Mycobacterial infections and/or subclinical hepatitis have also been mentioned as a possible factor in accounting for low CD4 T-cell counts in the Chinese population (16).

Limitations: This study has a small sample size and was hospital-based with most of the patients from Addis Ababa. This may not represent the general population with latent TB. But this study clearly showed that Ethiopia remains one of the high Latent TB burden countries in the world. Focusing on interventions against latent TB would contribute to reducing the incidence of TB in the country.

Conclusions: In this study, magnitude of latent TB was high which is consistent with previous studies conducted here in Ethiopia. There is currently no agreed gold standard for the diagnosis of LTBI. Both TST and QFT GIT have similar efficacy in diagnosis of latent TB in our study. Further studies of adults and children will be required to assess the effects of factors which may affect the results of both tests with age. The CD4, CD8, CD3 and CD4/CD8 ratio from this study was low, which is consistent with previous studies done in Ethiopia.

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