INTRODUCTION

People living with human immunodeficiency virus (PLWHIV) who also have latent tuberculosis infection (LTBI) are at a greater risk for developing tuberculosis (TB) than those without HIV infection (1,2). The World Health Organization (WHO) recommends screening and isoniazid preventive therapy (IPT) for PLWHIV co-infected with LTBI given a 62% reduction in incidence of TB (1.3). There are currently 2 types of tests used for diagnosing LTBI, the tuberculin skin test (TST) and interferon-gamma release assay (IGRA). Either TST or IGRA is recommended by the Centers for Disease Control and Prevention (CDC) for approving the commencement of IPT (4). TST is the test that is routinely available in resource-limited settings where IGRA is unavailable (1).

TST is less sensitive in immunocompromised persons than in their immunocompetent counterparts, and false-positive TST reactions have been associated with prior Bacillus Calmette–Guérin (BCG) vaccination and non-tuberculous mycobacterial (NTM) infections (5). Although IGRA are more sensitive and specific than TST in persons without HIV infection (6), a change from positive to negative results within both short and long-term periods were observed in PLWHIV with no or low TB risk (7,8). A meta-analysis of studies assessing TST and IGRA for LTBI screening among HIV-infected individuals demonstrated comparable performance between both tests (2). However, significant heterogeneity was noted among the studies included in this meta-analytic reports. In addition, the outcomes evaluated the predictive value of IGRA for active TB development and IGRA sensitivity in individuals using culture confirmation of active TB in comparison with TST (2). More longitudinal studies evaluating IPT efficacy based on positive TST, with or without IGRA testing in PLWHIV from high-endemic TB countries, are needed for evidence-based treatment strategies.

There has been increasing interest in identifying a clinical biomarker of response to IPT. Previous studies have reported a positive correlation between IGRA- assessed effector T-cell responses and the antigenic and the bacillary burden of Mycobacterium tuberculosis (9,10). Decreased T-cell responses after active TB and LTBI treatment have been demonstrated in non-HIV-infected individuals (9,11), however, to date, reports of changes in interferon-gamma (IFN-γ) levels after LTBI treatment remain controversial (12–14). Additionally, data regarding the long-term IFN-γ responses after IPT among HIV-infected patients from high-endemic TB settings are limited (15).

In Thailand, a TB prevalence of 171 cases per 100,000 individuals was estimated in 2014 (16). Guidelines for LTBI screening and treatment have not been established because of the absence of studies assessing the long-term efficacy of TB preventive strategies. The aim of the present study was to evaluate the efficacy of IGRA-...
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guided IPT in PLWHIV and to assess the IFN-γ responses after IPT in patients with LTBI in a high-endemic TB setting.

MATERIALS AND METHODS

Study design and setting: This cohort study was conducted from March 1, 2012 through February 29, 2016 among adult patients (aged ≥ 18 years) enrolled in HIV care at Thammasat University Hospital in central Thailand. The study was conducted in accordance with the amended Declaration of Helsinki and was approved by Ethics Committee of the Faculty of Medicine, Thammasat University. Informed consent was obtained from all patients before participating in the study.

Study protocol, definitions, and outcome measurement: All patients enrolled at the HIV clinic were screened for eligibility to participate during the first year of the 4-year study period. The exclusion criteria were: prior or currently active TB, history of LTBI treatment, or TST performed within the past year. Demographic data, BCG vaccination, history of TB contact, HIV clinical characteristics, and comorbidities were obtained by interview and reviewing medical records. Long-term alcohol use and smoking were defined as everyday use of any amount of the substances for at least 1 year prior to enrollment. Screening for active TB was performed based on symptoms and chest radiograph as recommended by WHO (1).

At enrollment, all participants received TST and IGRA tests. The TST was performed immediately after collection of the blood sample for the IGRA test, the QuantiFERON-TB Gold In-Tube Test (QFT-IT; Cellestis, Carnegie, Victoria, Australia). The interpretation of the results of QFT-IT were performed according to the manufacturer’s protocol (4). The level of IFN-γ was calculated by subtracting TB antigen response with nil response. Reversion of a QFT-IT test was defined as a positive baseline test that became negative during subsequent testing. In contrast, conversion of a QFT-IT test was defined as a change from negative to positive in at least one subsequent testing. For TST, 0.1 mL (5 tuberculin units) of purified protein derivative RT-23 (Tubersol; Connaught, Toronto, Canada) was administered intradermally to the inner forearm, and results were measured after 48–72 h by the ballpoint pen method. An induration of at least 5 mm was defined as TST reactivity. Based on initial these tests participants were categorized into 4 groups. Group 1 (QFT-IT-positive, TST-reactive), group 2 (QFT-IT-positive, TST-non-reactive), group 3 (QFT-IT-negative, TST-reactive), and group 4 (QFT-IT-negative, TST-non-reactive). Patients who developed symptoms suggestive of active TB underwent further investigations depending on the suspected site of infection, i.e., chest radiographs, sputum examination for acid-fast bacilli, and microbial cultures of expectorant, induced or bronchoalveolar lavage specimen for pulmonary TB. LTBI was treated by administering 300 mg isoniazid per day for 9 months. Adherence to IPT was monitored by standardized monthly pill counts (17). Combined antiretroviral therapy (ART) was initiated in patients with CD4 < 350 cells/μL according to the national guidelines. The primary outcome of this study was to assess the incidence of TB; the second outcome was to evaluate the all-cause mortality, HIV clinical outcomes, including, retention in care, change in CD4 counts, and HIV virological responses. Further, reversion and conversion of test results and changes in the IFN-γ level were assessed in QFT-IT-positive patients during the follow-up period.

Statistical analysis: Data analysis was performed using SPSS ver. 15 (Chicago, IL, USA). Pearson’s χ² or Fisher’s exact test was used to compare categorical data, when appropriate. Continuous variables were compared using the Mann-Whitney U-test or Kruskal-Wallis test based on the number of compared groups. Incidence of TB was calculated and compared using generalized linear models based on Poisson distribution. All P values were two-tailed; P < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the study cohort: The cohort comprised 150 patients with a median age of 40 years (range 18–65 years); of these, 79 (53%) patients were men, 119 (79%) had a CD4 count of ≥ 200 cells/μL, 109 (73%) had received prior BCG vaccination, 21 (14%) reported a history of TB exposure, and 113 (75%) were currently on ART of whom 109 (96%) reported HIV RNA suppression at the start of the study. There were no indeterminate QFT-IT results. Based on these tests the number of patients in each of 4 groups were as follows: 8 patients in group 1; 12 patients in group 2; 16 patients in group 3; and 114 patients in group 4. Most baseline characteristics were comparable among the groups, however, QFT-IT-positive, TST-non-reactive patients were predominantly women; QFT-IT-negative, TST-reactive patients were more likely to report long-term tobacco use (Table 1).

Fig. 1. Study flow. All groups received tuberculosis (TB) symptom screening at each visit and chest radiograph yearly and when developing compatible symptoms including fever, productive cough more than 2 weeks, weight loss, and night sweats. Groups 1 and 2 were offered isoniazid preventive therapy (IPT) at baseline and received annual QuantiFERON-TB Gold In-Tube Test (QFT-IT). Group 4 received annual tuberculin skin test (TST) and IPT if subsequent TST was reactive, pos, positive; neg, negative; R, reactive; NR, non-reactive; f/u, follow-up.
Tuberculosis and HIV outcomes: Majority of participants (95%) completed the 3-year follow-up period, and none reported new active TB exposure (Fig. 1). The incidence of active TB was significantly higher in the QFT-IT-positive, TST-non-reactive group (group 2) than in the other groups (2.78 vs. 0 cases/100 patient–years; Table 2). All-cause mortality, retention in care, median CD4, and changes in CD4 cell count, and rates of HIV RNA suppression for each follow-up year were not significantly different among the 4 groups. Sixteen of the 20 QFT-IT-positive patients (80%) from groups 1 and 2 accepted IPT, receiving at least 6 months of IPT (15 received isoniazid for 9 months, whereas 1 received only 6 months of the therapy due to 5-fold asymptomatic elevation of transaminases) with at least 95% pill count adherence. There were no reports of other adverse reactions from IPT. After the 3-year period, complete follow-up data were collected for 19 of the 20 (95%) QFT-IT-positive patients. One QFT-IT-positive, TST-non-reactive (group 2) patient who had refused IPT, subsequently developed active pulmonary TB, in contrast to the absence of TB cases in the other groups. Incident TB was significantly higher in patients who refused IPT than in those completing IPT (11.11 vs. 0 case/100 patient–years; $P < 0.001^*$).

Table 2. Characteristics of the 150 patients with human immunodeficiency virus infection stratified by baseline latent tuberculosis infection test results1)

| Characteristic                      | Group 1 (n = 8) | Group 2 (n = 12) | Group 3 (n = 16) | Group 4 (n = 114) | P   |
|-------------------------------------|-----------------|------------------|------------------|-------------------|-----|
| Age (yr): median (range)            | 42 (25–49)      | 45 (29–64)       | 41 (26–53)       | 39 (18–65)        | 0.31|
| Sex: men                            | 7 (88)          | 3 (25)           | 10 (63)          | 59 (52)           | 0.04*|
| Long-term tobacco smoking           | 1 (13)          | 0 (0)            | 7 (44)           | 15 (13)           | 0.04*|
| Long-term alcohol drinking          | 2 (25)          | 2 (17)           | 5 (31)           | 19 (17)           | 0.50|
| Prior BCG vaccination               | 6 (75)          | 9 (75)           | 12 (75)          | 82 (72)           | 0.99|
| History of tuberculosis contact     | 1 (13)          | 2 (17)           | 2 (13)           | 16 (14)           | 0.99|
| Diabetes mellitus                   | 0 (0)           | 1 (8)            | 1 (6)            | 4 (4)             | 0.75|
| Duration of HIV infection (mo)      | 30 (1–144)      | 45 (12–168)      | 54 (1–144)       | 56 (1–264)        | 0.85|
| CD4 count at enrollment (cells/µL) | 461 (242–600)   | 405 (113–1,148)  | 376 (9–914)      | 349 (8–1,290)     | 0.38|
| CD4% at enrollment median (range)   | 16 (7–27)       | 20 (11–35)       | 21 (1–30)        | 19 (1–40)         | 0.54|
| Receipt of antiretroviral therapy    | 4 (50)          | 11 (92)          | 14 (88)          | 84 (74)           | 0.12|
| HIV RNA suppression                 | 4 (50)          | 11 (92)          | 13 (81)          | 81 (71)           | 0.17|
| Baseline TST reaction size (mm)     | 14 (10–30)      | 0 (0–2)          | 9 (5–13)         | 0 (0–4)           | <0.001*|
| Baseline IFN-γ level (IU/mL) median (range) | 1.17 (0.36–9.88) | 0.82 (0.38–4.31) | 0.01 (0–0.87)–0.29 | 0.01 (0–0.31)–0.32 | <0.001*|

1) Data are in number (%) unless otherwise indicated.

Table 2. Tuberculosis and HIV outcomes during the 3-year study period1)

| Outcome                                      | Group 1 (n = 8) | Group 2 (n = 12) | Group 3 (n = 16) | Group 4 (n = 114) | P   |
|----------------------------------------------|-----------------|------------------|------------------|-------------------|-----|
| Tuberculosis outcomes (cases/100 patient–year) | 0               | 2.78             | 0                | 0                 | <0.001*|
| Completed active tuberculosis treatment      | NA              | 1/1 (100)        | NA               | NA                | –   |
| All-cause mortality (cases/100 patient–year) | 0               | 0                | 0                | 1.00              |     |

1) Data are in number (%) unless otherwise indicated.

* Statistically significant (< 0.05); QFT-IT+, positive QuantiFERON-TB Gold In-Tube Test; QFT-IT−, negative QuantiFERON-TB Gold In-Tube Test; TST-R, reactive tuberculin skin test; TST-NR, non-reactive tuberculin skin test; BCG, Bacillus Calmette-Guérin; IFN-γ, interferon-gamma.
differences between the 2 groups were not significant. in their IFN-γ study period but demonstrated a significant decrease of 3 patients (19% found in IFN-γ) not (group 4) did not have a reversion during the follow-up period. Neither TST conversion nor active TB case was detected for patients in this group during the follow-up period. This finding suggests the potential use of serial TST in monitoring LTBI status after initial negative TST and IGRA results among HIV-infected patients in resource-limited settings.

Additionally, this study demonstrated significant reduction in IFN-γ levels after IPT in patients with LTBI at 1 year as well as reduced levels between 1 and 2 years, although this difference was not significant. Changes in IFN-γ level among patients who refused IPT were also not significant. These findings suggest that frequent serial IGRA tests may be useful for monitoring IPT response, especially in cases where the drug resistance of M. tuberculosis is unknown. Other studies have reported similar responses of IFN-γ after LTBI treatment in both non-HIV-infected (11,13,14,18,19) and HIV-infected individuals (15). However, studies on non-HIV-infected patients have reported that the significant decrease of IFN-γ level occurred regardless of compliance to LTBI treatment (14,20). In a study from South Africa (14), QFT-IT was performed at a median of 9 days after TST. Considering that TST may falsely elevate the baseline level; (median change 0.74 IU/mL at 1-year and -1.50 IU/mL at 2-year from the initial level ≥1.2 IU/mL). The 3 QFT-IT-positive patients who declined IPT did not show QFT-IT reversion. The QFT-IT-positive patient who subsequently developed active TB after declining IPT demonstrated decreased IFN-γ levels from 0.76 IU/mL to 0.63 IU/mL at 1-year (after completing active TB treatment) and to 0.28 IU/mL at 2-year, that was considered reversion.

DISCUSSION

The major finding of this study was the effectiveness of QFT-IT-guided IPT in preventing TB. None of the QFT-IT positive patients who completed at least 6 months of IPT developed active TB during the 3-year follow-up period. The effectiveness of QFT-IT-guided LTBI treatment in this study was consistent with the results of a previous Norwegian study (15); however, the risk of TB exposure during the follow-up period in our cohort might be greater because of the higher prevalence of TB in the Thai population. The use of both QFT-IT and TST at the initial screening enabled us to categorize patient groups with concordant and discordant test results and allow for the comparative evaluation of test performance. Among the 20 patients with LTBI defined by QFT-IT-positive results, 12 (60%) were TST non-reactive. The incidence of active TB in these patients was significantly higher among those who refused IPT than those who accepted treatment. By using incident TB as the reference standard, these findings indicate that QFT-IT performed better than TST in identifying HIV-infected patients at-risk for developing active TB.

Subsequent LTBI testing is generally recommended in HIV-infected patients demonstrating negative results during initial screening with the rationale that the patient could subsequently develop TB or that low CD4 cell counts at entry into care could have confounded initial screening results (17). Considering the unknown benefit of serial IGRA testing and associated costs (8,15), we utilized TST as the subsequent test in patients with initial negative QFT-IT and non-reactive TST results (i.e., group 4). Neither TST conversion nor active TB case was detected for patients in this group during the follow-up period. This finding suggests the potential use of serial TST in monitoring LTBI status after initial negative TST and IGRA results among HIV-infected patients in resource-limited settings.
(23%; 15) or the non-HIV-infected cohort (5–42%) (11,13,14,18–20). The higher reversion rate in our study than in the Norwegian study could be because of our higher rate of compliance to LTBI treatment, lower median IFN-γ baseline level (1.05 IU/mL vs. 3.48 IU/mL), and a higher proportion of patients completing serial QFT-IT testing (95% vs. 52%) enabling more accurate assessment of IFN-γ responses. Compared to the studies on non-HIV-infected individuals (11,13,14,18–20), the higher reversion rate in this study could be explained by lower median baseline IFN-γ level and longer period between initial and subsequent tests (24 months vs. 4–9 months) after LTBI treatment that allowed detection of delayed reversion. In addition, most of the studies on non-HIV-infected persons did not assess adherence to LTBI treatment, which may have an effect on the IFN-γ response. Considering the effect of the initial IFN-γ level on reversion, utilizing the extent of IFN-γ decline to define IPT response instead of reversion at the time of retest may be more appropriate.

The limitations of our study findings are as follows: first, small sample size and necessitating larger cohort studies to confirm evidence-based treatment guidance using IFN-γ response and reversion in QFT-IT positive patients who either complete IPT or do not; second, we did not repeat QFT-IT in patients with initial negative QFT-IT result; instead, we used serial TST to monitor LTBI status per our protocol. Although the IFN-γ response could not be assessed among QFT-IT negative patients, this protocol was pragmatically-designed to be executed within our clinical care program to assess TB prevention in a resource-limited setting.

In conclusion, QFT-IT was effective in diagnosing LTBI and guiding IPT among HIV-infected patients in this high-endemic TB setting. Patients with QFT-IT negative results at screening can be safely monitored using TST concurrently with the commencement of ART. Among patients with LTBI, significant decline in IFN-γ response was observed after IPT. However, reversion was delayed or did not occur in those with high baseline IFN-γ levels. Further longitudinal studies with a larger sample size are needed to evaluate effectiveness of TST and IGRA-guided LTBI treatment, to determine appropriate extent of IFN-γ response and to determine the intervals between testing to define reversion after IPT in HIV-infected patients from an endemic TB setting.

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Conflict of interest None to declare.

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