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ORIGINAL ARTICLE

Role of QuantiFERON-TB-Gold In Tube assay for active and latent tuberculosis infection in investigation of tuberculosis outbreak in a university

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Background: Identification and monitoring of active tuberculosis (TB) and latent tuberculosis infection (LTBI) are the key steps to prevent transmission during a TB outbreak. The aim of this study was to evaluate the role of QuantiFERON-TB-Gold In Tube assay (QFT-GIT) in the investigation of active TB and LTBI cases during a TB outbreak in a university.
Methods: In this study, enrolled students and teachers were evaluated with chest radiograph, questionnaire, and QFT-GIT test. The diagnosis of active pulmonary TB was based on sputum studies and chest radiographs. The questionnaire, which covered demographic information, underlying diseases, and environmental exposures, was applied to assess the association of risk factors by multiple logistic regressions.

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Results: A total of 159 participants completed the study protocol. Positive QFT-GIT results were demonstrated in class A (75.7%; 25/33), class B (57.1%; 20/35), and class C (37.5%; 3/8) in institute 1; class D (17.3%; 8/46) in institute 2; and class E (3.1%; 1/32) in institute 3; but none among the (0/5) administrative officers, who comprised the control group. "Number of contact with active TB cases" was strongly associated and correlated with the prediction of a positive QFT-GIT result in multivariate analysis (odds ratio = 1.99; 95% confidence interval, 1.52—2.61; $p < 0.0001$). Seven cases progressed to active TB infection, all showing positive QFT-GIT results (100%; 7/7).
Conclusion: Inclusion of QFT-GIT may be helpful in controlling and monitoring of active TB and LTBI cases during an investigation of a TB outbreak. The finding demonstrated that the QFT-GIT test was useful in accurately identifying infected and uninfected students, permitting rapid intervention.

Introduction

Identification and monitoring of active tuberculosis (TB) infection and latent TB infection (LTBI) are the important steps of infection control to stop transmission during a TB outbreak, especially in a school setting where students live and study in crowded, enclosed spaces. Large-scale TB outbreaks have occurred in schools around the world.[1—4] Diagnosis of active pulmonary TB was based on clinical examination, chest radiography, acid-fast stain, a molecular method, and sputum culture.[5] Identification of LTBI was suggested by methods of tuberculin skin test (TST) and interferon-gamma (IFN-γ) around the world including Taiwan.[6] Until recently, TST was the available tool for the diagnosis of LTBI; however, it had major drawbacks, which included the possibility of obtaining false-positive results because of cross-reactivity with nontuberculous mycobacteria or with the bacillus Calmette—Guerin (BCG) vaccination that had been used in Taiwan and many countries.[5] QuantiFERON-TB Gold In Tube assay (QFT-GIT; Cellestis Limited, Carnegie, Victoria, Australia) was an immunologic assay that measured the IFN-γ released by T lymphocytes sensitized with the *Mycobacterium tuberculosis*-specific antigens ESAT-6 and CFP-10. The comparable sensitivity but higher specificity of this assay (in comparison with TST) had been known to aid in the diagnosis of both active TB infection and LTBI.[7,8]

Although a higher incidence of TB infection was reported in Taiwan, the prevalence of LTBI in the community was uncertain.[9] Our study was designed to assess the active TB infection and LTBI burden in BCG-vaccinated students using QFT-GIT and to determine the environmental and lifestyle factors that predisposed certain individuals to the infection. This study demonstrated the role of the QFT-GIT assay in detecting TB infection, including active cases and LTBI, during a TB outbreak in a university in Taiwan.

Materials and methods

Study populations

In November 2010, a 22-year-old female student suffered from fever and cough, and her chest radiograph was compatible with a primary TB complex infection. *M. tuberculosis* was isolated from the sputum culture. The following year, two of her classmates were confirmed to have active pulmonary TB infection. An immediate contact investigation (chest radiograph, questionnaires, QFT-GIT) was performed for all of the index case's classmates and for the other students in different institutes sharing the same classroom in this university. Closer contact was defined as exposure of 8 hours per day, or more than 40 hours in the same environment with transmissible active TB cases according to the definition of the Centers for Disease Control (CDC) in Taiwan.[6] Students who had close contact with the index case for 8 months were screened for TB infection and LTBI. The university environment was assessed with ventilation evaluation. To prioritize students for TB screening, the study was conducted among the students to identify the factors associated with close contact with the index case as per the criteria of the CDC in Taiwan.[5] The index patient was interviewed about her personal, social, and class activities during the school deployment. A questionnaire was designed to collect information on potential exposure factors among the study participants. All personnel had been evaluated using the questionnaire and blood examination of QFT-GIT. Individuals with positive QFT-GIT results were referred to hospitals. History taking, physical examination, acid-fast stain, culture, and polymerase chain reaction (PCR) for tuberculosis were evaluated if an abnormal finding of chest radiography was noted. Individuals with negative QFT-GIT results were followed up. The flow diagram for this study is shown in Fig. 1. We examined all participants who had positive QFT-GIT results with chest radiograph (CXR) during the 1st year. We checked for the presence of TB infection using sputum acid-fast bacilli (AFB) culture, and PCR in patients whose CXR indicated infiltration and who had a cough. The CXR, AFB, and PCR for sputum were interpreted by other investigators. The investigators who read the final CXR and took the patients' history to determine the patient's TB status were blinded to the QFT-GIT results.

AFB microscopy and culture

For acid-fast staining, decontaminated sputum was fixed onto a slide, and the number of AFB per field for an average

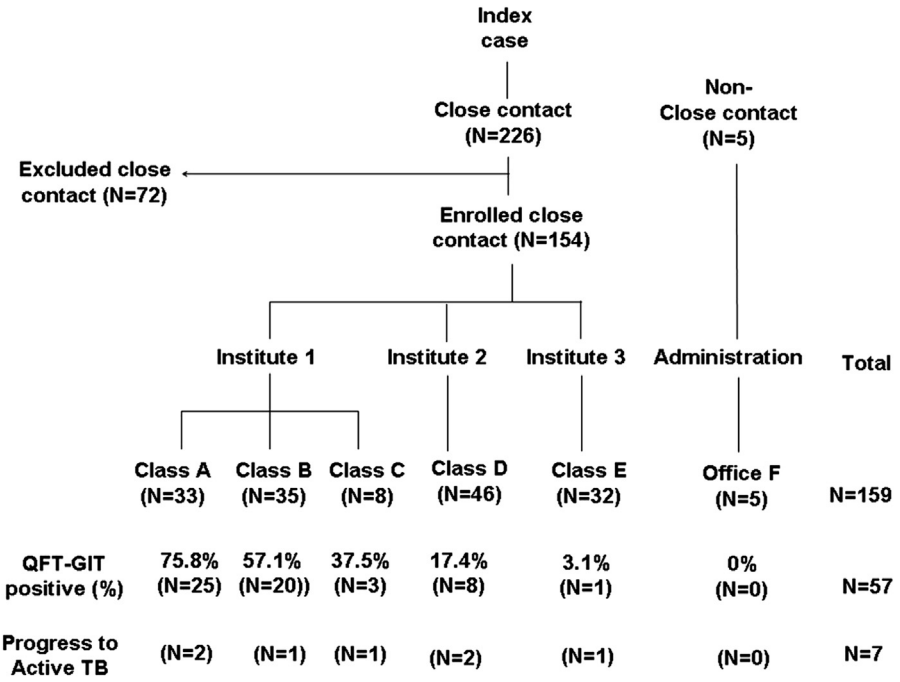

Figure 1. Flow diagram with results of QFT-GIT and progressed to active pulmonary TB of enrolled participators.

of 50 fields per sample was counted. Based on repeated counting on separate days, the results of this procedure are reproducible. TB culture was performed by plating homogenized-decontaminated sputum on the Lowenstein—Jensen medium and incubating it for 3—4 weeks at 37°C in 5% CO_2.[10]

PCR for *M. tuberculosis*

We performed PCR for *M. tuberculosis* DNA as previously described. We then electrophoresed the PCR product on polyacrylamide gels and confirmed its identity with a previously described standard method.[11]

Demographic data, lifestyle, and risk factors evaluated by questionnaire

The questionnaire, which was designed to include demographic data, lifestyle, and risk factors for LTBI and TB infection, was administered among the students after they had given informed consent to participate in the study. The demographic data—which included age, body weight, height, education, family history, civil status (marriage), and location of living quarters—were studied. The personal medical history, symptom/signs, lifestyle habits such as smoking and drinking were also evaluated. The previous work environment and number of contacts with active TB patients were also surveyed.

QFT-GIT assay

The QFT-GIT assay detached INF-γ, which was released from sensitized lymphocytes upon stimulation with Early-Secreted Antigen, Culture—Filtrate Protein-10, and Protein TB (p4) were determined as recommended by the manufacturer. The plasma IFN-γ concentration was measured using a commercial QFT-GIT enzyme-linked immunosorbent assay and was determined as negative and positive (cutoff at 0.35 IU/ml) by the manufacturer's software.[8]

Statistical analyses

Student's *t* test and Chi-square measures were performed to compare the different risk factors and relevant data between QFT-GIT. Data were expressed as mean $\pm$ standard deviation (SD). Multivariate analyses of SAS and MedCalc (9.1.3, Mariakerke, Belgium) were performed to compare the differences in the adjusted groups. If significant results were found, these variables were examined by univariate analyses. A p value < 0.05 was considered statistically significant.

Results

Prevalence of LTBI and progression to active TB in enrolled close contacts

A total of 226 students who were classified to have close contact based on the criteria of the CDC and five officers as control individuals were enrolled in the study. Immediate contact investigations (chest radiograph, questionnaire, QFT) were performed for all those who had closer contact with the index case and two more students who had active pulmonary TB in different institutes sharing the same classroom of this university (Fig. 1). A total of 72 students refused to participate and were excluded from the study. In all, 154 students in three different institutes and five administration officers as controls were enrolled. They

Table 1 Univariate analysis of risk factor for a positive QFT-GIT assay

Variables	QFT-GIT (+)		QFT-GIT (−)		*p*
	n	Mean ± SD (%)	*n*	Mean ± SD (%)	
Age,[a] *n* (mean ± SD)	57	24.30 ± 10.50	102	29.20 ± 12.90	0.0166
Height,[a] *n* (mean ± SD)	57	166.00 ± 6.00	102	164.80 ± 7.70	0.2840
Weight,[a] *n* (mean ± SD)	57	57.80 ± 15.00	102	59.60 ± 10.80	0.4288
BMI,[a] *n* (mean ± SD)	57	20.80 ± 4.90	102	21.90 ± 3.20	0.1728
Class h/d,[a] *n* (mean ± SD)	57	6.32 ± 1.93	102	6.72 ± 2.37	0.3002
Number of contact with TB patients,[a] *n* (mean ± SD)	57	4.20 ± 1.40	102	2.50 ± 1.40	<0.0001*
Sex,[b] *n* (%)					0.3134
Male	23	(41.10)	34	(33.00)	
Female	34	(58.90)	68	(67.00)	
History of old TB,[b] *n* (%)	1	(1.80)	0	(0.00)	0.3590
Family history of TB,[b] *n* (%)	6	(10.70)	6	(6.00)	0.3516
BCG vaccination[b]	51	(91.10)	92	(92.90)	0.7576
Cancer,[b] *n* (%)	0	(0.00)	1	(0.10)	> 0.99
Diabetes,[b] *n* (%)	3	(5.40)	1	(1.00)	0.1322
Fatigue for 2−3 mo,[b] *n* (%)	5	(8.90)	10	(10.00)	> 0.99
Weight loss,[b] *n* (%)	0	(0.00)	2	(2.00)	0.5368
Cough up phlegm,[b] *n* (%)	6	(10.70)	8	(8.00)	0.5694
Chest pain,[b] *n* (%)	4	(7.10)	6	(6.10)	0.7484
Difficult breathing,[b] *n* (%)	2	(3.60)	6	(6.10)	0.7114
Hemoptysis,[b] *n* (%)	0	(0.00)	1	(1.00)	> 0.99
Afternoon fever,[b] *n* (%)	0	(0.00)	3	(0.30)	0.5533
Smoking,[b] *n* (%)	6	(10.70)	15	(15.00)	0.4519
Drinking,[b] *n* (%)	9	(16.10)	8	(8.00)	0.1207

[a] *t*-Test.
[b] Chi-square test.
BCG = bacille Calmette−Guerin; BMI = body mass index; Number of contact with TB patients = exposure with numbers of active TB cases.
* Statistically significant ($p < 0.05$).

were designated as follows: class A ($n = 33$), class B ($n = 35$), and class C ($n = 8$) in institute 1; class D ($n = 46$) in institute 2; class E ($n = 32$) in institute 3; and administration officers ($n = 5$). Of this total, a positive QFT-GIT result was obtained in 35.8% (57/159). The higher positive QFT-GIT result was found in different classes of students who had closer contact. It was demonstrated in 75.7% (25/33) of class A, 57.1% (20/35) of class B, 37.5% (3/8) of class C, 17.3% (8/46) of class D, 3.1% (1/32) of class E, and 0% in the control group consisting of administrative officers (0/5). During the investigation of this outbreak, two more students from different classes of institute 1, two students in institute 2, and one student in institute 3 with higher positive QFT-GIT result progressed to active pulmonary TB infection (Fig. 1). The results of QFT-GIT and enrolled participants who progressed to active pulmonary TB are shown in Fig. 1.

Determination of risk factors of LTBI and active pulmonary TB by demographic data, lifestyle, and environment

The questionnaires, which were completed by all study participants after giving informed consent and undergoing a blood test, were analyzed. The demographic data, lifestyle, symptoms/signs, underlying diseases, and environment between the groups of participants who had positive QFT-GIT and negative QFT-GIT results were compared. The item of "number of contact with active TB cases" was the only associated risk factor and correlated with prediction of a positive QFT-GIT result in a univariate analysis for LTBI (4.2 ± 1.4 vs. 2.5 ± 1.4, $p < 0.0001$). The demographic and clinical characteristics of the students with QFT-GIT result are shown in Table 1. After adjusting for factors including age in the study with multivariate analysis, "number of contacts with active TB patients" was still the predominant risk factor that was significantly associated with a positive QFT-GIT result (odds ratio = 1.99; 95% confidence interval, 1.52−2.61; $p < 0.0001$) (Table 2).

Correlation between progression of active tuberculosis and LTBI of close contacts

The number of contacts with active TB cases was the risk factor that was significantly associated with a positive QFT-GIT result and progress to active pulmonary TB. From the results of positive QFT-GIT associated with close contact with number of active pulmonary TB cases, all the students who eventually progressed to active TB infection were demonstrated to have positive QFT-GIT results (7/7, 100%).

Table 2 Multivariate analysis of risk factor for a positive QFT-GIT assay

Variables	Adjusted		p
	OR	95% CI	
Number of contact with active TB cases,[a] *n* (mean ± SD)	1.99	(1.52–2.61)	<0.0001*

[a] Logistic regression analysis with stepwise method.
Adjusted variables: age.
* Statistically significant ($p < 0.05$).
CI = confidence interval; OR = odds ratio.

The relationship between TB incidence and LTBI in an outbreak stemming from crowded spaces had been demonstrated. The strong correlation of the prevalence rate of LTBI with the number of contacts with active TB cases was also found (Fig. 1 and Table 2).

Discussion

This study was designed to evaluate the effectiveness of the QFT-GIT assay in detecting LTBI ($n = 57$) and active TB ($n = 8$) cases during a TB outbreak in a Taiwanese university. Of the index case and seven students' progression to active TB infection, the QFT-GIT assay showed 100% positive results. Close contacts with positive QFT assay ranged from 3.1% to 75.1% compared with nonclose contact of 0%. The number of contacts with active TB cases was an independent predictor of a positive QFT-GIT result in multivariate analysis. These findings demonstrated that the QFT-GIT test was extremely useful in accurately identifying infected and uninfected students, permitting rapid intervention and control in outbreak evaluation.

The diagnosis of TB was based on history taking and physical examination, chest radiography, and the TST.[5] For diagnosis of LTBI, TST had been used for years as an aid in diagnosing LTBI after an intradermal injection of purified protein derivative. The QFT-GIT is an immunologic assay and had been demonstrated to have a good specificity and sensitivity for the diagnosis of both active TB infection and LTBI. The QFT-GIT was approved by the Food and Drug Administration as an aid for detecting LTBI in 2001.[8,12] From the previous study of a CDC-sponsored multicenter trial, QFT-GIT and TST results were moderately concordant (overall kappa value = 0.60). The level of concordance was adversely affected by prior BCG vaccination, immune reactivity to nontuberculous mycobacteria, and a prior positive TST result.[7] Diel et al[13] had reported a stronger association of the level of exposure to patients with active TB of QFT-GIT results compared with TST. The QFT-GIT assay had several advantages: it was not affected by BCG vaccination, testing was easy to administer because it requires only one visit, and the measurements were not subject to interpretation bias.[13]

The QFT-GIT can aid in detecting *M. tuberculosis* infections among certain populations who are at increased risk for LTBI. These populations include recent immigrants from high-prevalence countries where tuberculosis case rates are ≥30/100,000, injection-drug users, residents and employees of prisons and jails, and healthcare workers.[14] Guidelines on the investigation and prevention of TB through contact investigations were recently suggested by the National Tuberculosis Controllers Association and the CDC of the United States.[15]

There have been strong and sufficient lines of evidence demonstrating the association between ventilation, air movements in buildings, and the spread of infectious diseases such as TB. However, there has been insufficient data to specify and quantify the minimum ventilation requirements in schools in relation to the spread of infectious diseases via the airborne route.[16] In this study, the classroom located in crowded basement had poor ventilation condition, which might be an important factor for this outbreak.

The limitations of our study were the shorter duration of follow-up in LTBI and the lack of cases to receive antituberculosis chemotherapy. The exclusion of about one-third ($n = 72$) of those who had close contact with the index and active TB cases might be one defect in this TB outbreak investigation (because of a lack of written informed consent). This TB outbreak finally was controlled with the intervention of infection control by the university. No new pulmonary TB cases were found during the 12-month follow-up. A longer follow-up and monitoring period for students with higher positive QFT-GIT results should be performed for the evaluation of progression to new TB infection.

In conclusion, the inclusion of the QFT-GIT assay in the investigation of a TB outbreak may be helpful for controlling and monitoring of active TB infection and LTBI cases. Methods for active and LTBI cases identification, rapid diagnosis, and tracking of TB infection are required to allow the authorities to take the appropriate management strategies to alleviate the morbidity and mortality caused by TB during an outbreak in the community. The combination of close contacts in active pulmonary TB with the QFT-GIT test might be used initially in screening for LTBI rather than using TST in higher prevalence BCG-vaccinated areas, including Taiwan.

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