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Comparison of QuantiFERON TB-G-test to TST for detecting latent tuberculosis infection in a high-incidence area containing BCG-vaccinated population

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Keywords
Latent Tuberculosis infection (LTBI), QuantiFERON-TB Gold In-Tube test (QFT), tuberculin skin test (TST)

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Abstract

Objective Until recently, the only tool for detection of latent tuberculosis infection (LTBI) was the tuberculin skin test (TST). QuantiFERON-TB Gold In-Tube test (QFT) is a promising *in vitro* diagnostic test for LTBI that has potential advantages over the TST. In this study we aimed to compare QFT with TST for diagnosis of LTBI.
Patients and methods A total of 186 BCG-vaccinated subjects enrolled in study. They underwent TST and QFT assay. They divided in two groups. Group 1 includes individuals who were at low risk for exposure to *M. tuberculosis* (LRG) and Group 2 includes individuals who were likely to have been exposed to *M. tuberculosis* infections (HRG).
Results Overall agreement between QFT and TST was 89.3% (kappa = 0.052). In LRG, agreement between the two tests was 52.6% (95% confidence interval, 44–60%) with κ-values of 0.019. In HRG agreement between the two tests was 63.2% (95% confidence interval, 42–84%) with κ-values of 0.28.
Conclusion In conclusion, the QFT assay showed acceptable results for determining latent *M. tuberculosis* infection in vaccinated population. The decision to select QFT over TST will depend on the population, purpose of testing and resource availability.

Introduction

The World Health Organization has estimated that approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* [1,2]. This large pool of individuals with latent infection poses a major hurdle for global tuberculosis (TB) control efforts. Between eight and nine million people develop TB disease each year, and about two million die from TB every year [1,3,4].

The tuberculin skin test (TST) is used for the identification of latent tuberculosis infection (LTBI) [5]. Although widely used, it cannot be trusted as a 'gold standard' because of the variability of interpretation and false positives and negatives [6]. Placement of the purified protein derivative (PPD), subjective reading of the results, and the unwillingness of individuals to return for test interpretation are responsible for many problems associated with its use [7].

In addition, interpretation of a TST result is often complicated in individuals vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) or exposed to environmental mycobacteria because of the occurrence of cross-reactive (false–positive) immune responses to antigens present in tuberculin (PPD) which are shared by those of non-tuberculous mycobacteria (NTM) and BCG [5].

A novel diagnostic assay for detection of LTBI which is more specific than the TST and the result of which would not be affected by previous BCG vaccination would be of great practical use, especially in countries where TB is endemic [5].

The QuantiFERON-TB Gold In-Tube test (QFT) detects the *in vitro* cell-mediated immune response to *M. tuberculosis* infection by measuring IFN-γ in whole blood that was incubated with *M. tuberculosis* antigen [early secretory antigenic target – 6 (ESAT-6), culture filtrate protein-10 (CFP-10) and another peptide from the TB antigen TB7.7 (Rv2654)]. An enzyme-linked immunosorbent assay (ELISA) detects the amount of IFN-γ produced by the T cells [6]. Because these proteins are absent from all BCG vaccine strains and from commonly encountered *NTM* except *M. kansasii, M. szulgai,* and *M. marinum* [8], QFT is expected to be more specific for *M. tuberculosis* than tests that use tuberculin PPD as the antigen [9].

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In this study we set out to compare performance of QFT (Cellestis Limited, Carnegie, Victoria, Australia), used for detection of latent *M. tuberculosis* infection, with TST in two groups including subjects without any known risk factors for *M. tuberculosis* infection and individuals with known risk factors for *M. tuberculosis* infection. Both groups were BCG-vaccinated.

Patients and methods

Study population

We recruited and enrolled 186 individuals for this study from April through December 2006 at the Laboratory of Pasteur Institute of Iran. Neither immunocompromised nor pregnant individuals participated in this study. All study enrollees provided written consent forms and answered questions that included details about age, past medical history, prior vaccination with BCG and prior TB exposure information. Individuals were 17 or older and were individuals required to take a TB test for employment. The study population consisted of 156 men (83.87%) and 30 women (16.13%) with mean age 28.40 ± 9.10 years old (ranged from 17 to 68). Based on responses to the form's questions and a review of medical records, we divided participants into two groups. Group 1, individuals who were considered as 'low risk group (LRG)' included participants who referred in order to pre-employment screening for TB by TST with no known risk factors for *M. tuberculosis* infection (previous exposure to a known index case or previous history of TB). Group 2 'high risk group (HRG)' included individuals with known risk factors for *M. tuberculosis* infection. All of the participants had received BCG vaccination according to schedule of Iranian immunization programme at birth.

Sample collection and TST

From each subject a 3-mL heparinized blood sample was collected by venipuncture for the QFT assay. For the TST, 0.1 mL of tuberculin PPD (Vaccine and serum Res. Institute I.R. Iran; equivalent to about five tuberculin units of PPD solution) was injected intradermally into the volar aspect of the forearm, and the transverse induration diameter was measured 72 hours later. The positive interpretation of a TST is an area of induration of ≥10 mm in individuals.

QFT-TB test

IFN-γ responses to ESAT-6, CFP-10 and TB7.7 (Rv2654) were measured by the QFT assay, as per the manufacturer's instructions (Cellestis Limited, Carnegie, Victoria, Australia). The QFT assay involved two stages: (1) incubation of whole blood with antigens; and (2) measurement of IFN-γ production in harvested plasma by ELISA. Venous blood was directly collected into three 1-mL heparin-containing tubes. One tube contained only heparin as negative (nil) control, another also contained the T-cell mitogen phytohaemagglutinin as positive control, and the third tube had overlapping peptides representing the entire sequences of ESAT-6 and CFP-10 and another peptide from the TB antigen TB7.7 (Rv2654). Within 1 hour of blood draw, the tubes were incubated at 37°C. After 20 hours of incubation, the tubes were centrifuged and plasma was harvested and frozen at –20 C until the ELISA was performed. The amount of IFN-γ was quantified using the QFT ELISA. The ELISA readout was analysed using the QFT software. IFN-γ-values [International Units (IU) per mL] for TB-specific antigens and mitogen were corrected for background by subtracting the value obtained for the respective negative control.

As recommended by the manufacturer and used in previous studies [10–13], an IFN-γ ≥0.35 IU mL^{-1} for (TB antigens – Negative control) was considered indicative of TB infection. For a QFT result to be valid, the (Mitogen – Negative control) must be ≥0.5 IU mL^{-1} and/or (TB antigens – Negative control) must be ≥0.35 IU mL^{-1}. All assays were deemed valid, and met the internal quality standards. No indeterminate results were reported.

Statistical analysis

Information from the questionnaires, TST and QFT results were entered into SPSS version 11.5. Agreement between the results of the TST and QFT tests was assessed by using κ coefficients, for both LRG and HRG. Kappa values below 0.4 indicate weak correlation; values of 0.41–0.60 indicate good agreement and values above 0.6 strong agreement [14]. Logistical regression was used to estimate odds ratios (ORs) of positive responses to age and sex. A *P*-value of <0.05 was considered significant. The sensitivity, specificity of the QFT were calculated by comparing LRG with HRG.

Results

Both the QFT and the PPD skin tests were administered to 186 individuals. Fourteen cases (7.5%) were QFT-positive and 172 cases (92.5%) were QFT-negative. Increasing age was a significant risk factor for QFT positivity (OR = 1.09, $P < 0.0008$), but sex didn't show any relation to QFT positivity.

Of our subjects, 89.24% (166) were in LRG and 10.76% (20) were in HRG. Mean age of LRG members and HRG members was 27.6 ± 8.6 and 35.1 ± 10.4 years respectively.

A total of 176 (156 LRG and 20 HRG) from these 186 subjects returned for PPD interpretation. Eighty-five subjects (48.3%) were PPD positive and 91 subjects (51.7%) were PPD negative. PPD was ranged between 0 and 36 mm with mean 9.7 ± 7.5 mm.

Eighty-six (48.86%) cases were negative by both assays, and nine (5.12%) cases were positive by both methods. Five patients (2.84%) were positive by QFT and negative by TST, and 76 (43.18) individuals were negative by QFT and positive by TST. Overall agreement of the QFT compared with the TST was 89.3% (with κ-values of 0.052).

In LRG, 48.71% (76 of 156) were both TST- and QFT-negative. Six subjects (3.85%) were both TST- and QFT-positive, five subjects (3.21%) were TST-negative and QFT-positive and 69 subjects (44.23%) were TST-positive but QFT-negative. This gave an agreement between the two tests in the low-exposure group of 52.6% (95% confidence interval, 44–60%) with κ-values of 0.019.

In HRG, 15% (three of 20) were positive in both tests, and 50% (10) were negative in both tests. Seven subjects (35%) were TST-positive and QFT-negative, and no subject had the inverse profile. In the high-exposure group, this gave an agreement between the two tests of 63.2% (95% confidence interval, 42–84%) with κ-values of 0.28. Comparison between TST and QFT positive results in two groups was summarized in Table 1. Agreement between QFT and TST results was shown in Fig. 1.

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Table 1 QFT and TST results in HRG and LRG

Exposure group	Total *n*	TST-positive *n* (%)	QFT-positive *n* (%)
High	20	10 (50)	3 (15)
Low	156	75 (40.08)	11 (7.05)

TST ≥ 10 mm.
TST, tuberculin skin test; QFT, QuantiFERON TB Gold In-tube test. HRG, high risk group; LRG, low risk group.

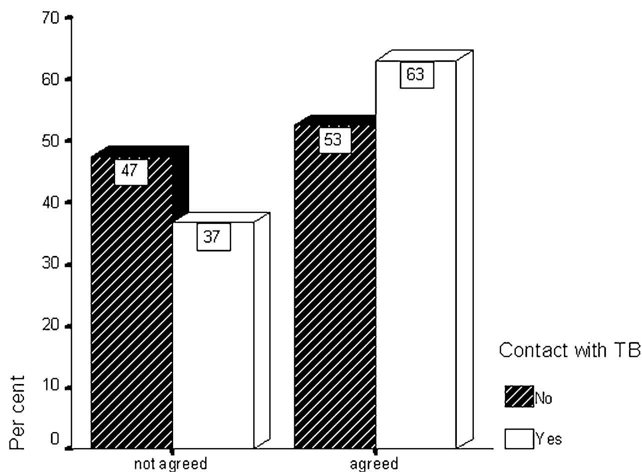

Figure 1 Agreement between QFT and TST results. TST, tuberculin skin test; QFT, QuantiFERON TB Gold In-tube test; TB, tuberculosis.

Discussion

Mycobacterium tuberculosis is a major global public health problem. The detection and monitoring of *M. tuberculosis* infections are essential to controlling its spread. Contemporary estimates argue that one-third of the earth's population are already infected, with the majority suffering from a latent form of infection [6].

Significant impediments to global tuberculosis control include limitations of current diagnostic tests [15]. Identification of *M. tuberculosis*-infected individuals is especially difficult in tropical environments, where BCG coverage is high and environmental mycobacteria are commonly encountered [16]. Until recently, the only tool available to LTBI was the TST. Although the TST is useful in clinical practice, it has several known limitations, including variable specificity, cross-reactivity with BCG vaccine and NTM infection, and problems with reliability [17,18]. So new tests are required for the identification of *M. tuberculosis*-infected individuals especially in tropical environments [16].

IFN-γ assays utilizing ESAT-6 and CFP-10 either as recombinant antigens (5,12) or mixtures of overlapping peptides [11,19] have been shown to be significantly more specific than TST [19]. In addition, these assays offer several advantages over TST, including the need for only a single patient contact, elimination of subjectivity in administering the test or reading the result, and the availability of test results within 24 hours. QFT have been studied in the general population in several countries including India, Korea, Italy, Denmark, the Netherlands and Japan [5,11,12,20–22].

Some studies showed that QFT assay is a better indicator of the risk of *M. tuberculosis* infection than TST in a BCG-vaccinated population [21,23]. Mazurek *et al*. reported that in those being screened for LTBI; the QFT assay was comparable with the TST in its ability to detect LTBI, and was less affected by BCG vaccination and discriminated responses as a result of *non-tuberculous mycobacteria* [24]. Taggart *et al*. demonstrated that 41.4% of healthy BCG-vaccinated individuals have positive results by the QuantiFERON-TB assay. QFT testing in low-risk group resulted in an agreement of 96.8%, a sensitivity of 50%, and a specificity of 98.4% compared with TST results [6]. In our study the overall agreement between TST and QFT assay was 89.3%(with κ-values of 0.052). This overall agreement between TST and QFT assay with low κ are consistent with and support the findings by other studies that have reported overall agreements of 81.4–95.0% [4,10,12,21].

Several studies have demonstrated discordance between TST and QFT results especially the type that TST was positive but QFT was negative. A recent study from South Africa found that among those with large TST reaction (≥15 mm), approximately one-third were negative by QFT [25]. In a study from India, 11% of individuals with positive TST (at least 15 mm) were negative by QFT [10]. In our study, 43.18% of our subjects were positive by TST but negative by QFT. It probably was due to prior BCG vaccination. Such discordance could also be due to false positive TST or it is plausible that QFT are less sensitive than TST in detecting LTBI. Besides in high incidence areas there are several factors that might modulate immune responses such as malnutrition, BCG vaccination, helminths and tropical infections that impact the T-helper (Th) 1/Th2 immune balance [26]. The other possibility is that the QFT, which relies on the presence of antigen-dependent immediate effector T-cells, is detecting current or more active infection, while the TST, which can detect central memory T-cell responses, is detecting past, dormant or resolved infection [27].

Discordance of the reverse type (TST-negative but QFT-positive) has also been documented, but it's largely unexplained [10,16,28].

In our subject increasing the TST cut-off from 10 mm to 15 mm resulted in better concordance between the results of TST and QFT in HRG population (half of TST-positive subjects were also QFT-positive). But with this cut-off in LRG group only approximately one-eighth of cases were positive with both tests. It suggested that QFT was able to identify those with TST responses in this range that were truly infected with MTB.

In conclusion, the QFT assay showed acceptable results for determining latent *M. tuberculosis* infection in vaccinated population. Although TST and QFT assay appear comparable, they have different performance and operational characteristics; therefore, the decision to select one test over the other will depend on the population, purpose of testing and resource availability. Larger studies are needed to compare the performance of the QFT assay with TST in patients with latent LTBI.

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