Original Article

Comparison of the Tuberculin Skin Test and the Quantiferon Test for Latent Mycobacterium tuberculosis Infections in Health Care Workers in Turkey

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SUMMARY: The aim of this study was to compare the efficacy of the tuberculin skin test (TST) and the quantiferon test (QFT) for detecting latent tuberculosis infection (LTBI) in health care workers (HCWs). Seventy-six participants who were working in Duzce University Hospital, where tuberculosis patients were being treated, were included in the study. TST was performed according to the Mantoux technique. QFT was performed in accordance with the manufacturer’s instructions. A positive TST result was defined as an induration diameter of ≥15 mm. TSTs were positive in 41 of 76 participants (53.9%) and QFT was positive in 65 of 76 participants (85.5%). There was a significant difference between the numbers of QFT-positive and TST-positive cases \( (P = 0.02) \). When the induration diameter of TST was ≥20 mm, QFT positivity was 100%. Multivariate analysis revealed that there was a significant correlation between the percentage of patients with QFT positivity and the induration diameter of TST \( (P = 0.009) \). QFT thus seems to be more effective for LTBI diagnosis than TST. However, large-scale trials including quantitative measurement of QFT in subgroups taking into account the division where HCWs are employed and the different results of TST might clarify the usefulness of QFT in LTBI diagnosis.

INTRODUCTION

The World Health Organization declared tuberculosis (TB) a global public health emergency in 1993. Although 95% of cases and 97% of all deaths occur in highly endemic areas, the disease continues to be a problem in industrialized countries as well, mostly in immigrant populations, in elderly individuals whose latent infection is reactivated, and in local outbreaks (1). Furthermore, there are other risk groups, such as intravenous drug users, patients with end-stage renal disease, human immunodeficiency virus (HIV)-positive patients, the homeless and health care workers (HCWs) (2). Chemotherapy to prevent latent Mycobacterium tuberculosis infection from progressing to overt disease is important in the TB elimination strategies of many countries. The tuberculin skin test (TST) has been used for years as an aid in diagnosing latent tuberculosis infection (LTBI). However, TST is neither 100% sensitive nor 100% specific, and an average of 20-25% of patients with active TB do not react to the purified protein derivative used in this test (3). Furthermore, the TST has other drawbacks, including the need for a return visit to allow reading, problems in interpretation due to the booster effect, the variability inherent in its application and reading, and modulations of the skin response due to underlying illness, immunosuppression, or cross-reactivity in Bacille Calmette-Guérin (BCG)-vaccinated people (4,5). Also, a positive tuberculin test will revert to negative unless restimulated by new aerosol inocula or persisting infection. In one tuberculin survey, 8.1% of positive reactors reverted to true negative when tested 1 year later (2).

Baseline 2-step TST is recommended for HCWs to identify cases of the boosting phenomenon and to track the risk of acquiring occupational TB. However, the 2-step TST has been shown to be insufficient to identify all cases of the boosting phenomenon in older adults and refugees (6,7).

In 2001, QuantiFERON®-TB (QFT) (Cellestis, Ltd., Victoria, Australia) was approved by the Food and Drug Administration as an aid for detecting LTBI. Principally, this test involves detection and quantitation of the cytokine gamma interferon (INF-\( \gamma \)) produced by T lymphocytes stimulated with purified protein derivatives (PPDs) obtained from either M. tuberculosis (human), M. avium (avian), or M. bovis (bovine) (8,9). QFT seems to overcome some of the drawbacks of TST performed with the standard Mantoux technique, because there is no requirement for patients to return to have their tests read, the results are less open to subjective interpretation, and QFT does not boost anamnestic immune responses. Furthermore, QFT is less affected by BCG vaccination and has better sensitivity and specificity than TST (8,10,11), and the results can be obtained more quickly than by TST. In addition, the positive control included in QFT helps to exclude false negative results related to immunosuppression. The drawbacks of QFT are that it is more expensive and more difficult to perform. However, the total cost of TST screening itself may be underestimated (10,12-15).

Although there was not any agreement in this subject, some authors said that QFT was usable in diagnosis of LTBI (10,12-15).

In this study, we aimed to compare the efficacy of QFT and TST for detecting LTBI in HCWs in Turkey, who are known to be at risk for LTBI due to the high incidences of LTBI and BCG vaccination in this country (11).
MATERIALS AND METHODS

Study population: This study was conducted at Duze University Medical School Hospital, which is located in a rural area in the northwest part of Turkey, between June 15 and August 15 of 2005. This university hospital was established in 1998, and clinical services were started the following year. Participants in the study consisted of the 76 out of 150 hospital staff who provided their informed consent. All of the participants underwent a TST in 2002. The ones who were TST-negative in the first test underwent a second TST test (The 2-step TST). The boosting phenomenon was not observed in any of these participants.

Seventy-six hospital employees (21 doctors, 19 nurses, 22 medical receptionists, 9 custodians, and 5 laboratory staff members) were recruited from the different clinics (chest diseases, infectious diseases, pediatric diseases, internal medicine) and laboratory (microbiology laboratory) in which TB patients were being followed up. The receptionists and custodians had direct contact with patients. After providing written informed consent, all volunteers completed a detailed questionnaire about possible risk factors for exposure to M. tuberculosis. They also were asked to indicate whether they had received the BCG vaccination and to report details of any contact with people with TB, the presence of underlying illnesses, medication, history of previous TB or anti-TB treatment, and their age, birth date and occupation.

Skin testing: Prior to enrollment, all subjects were skin tested with 0.1 ml of 5-TU (tuberculin units) PPD injected intradermally according to the Mantoux technique. All TSTs were performed and read by a chest-disease specialist. All readings were performed with the palpation and ballpoint methods along two axes of the forearm, after 48 h. The results of the TST were considered positive when the induration diameter was ≥15 mm according to both the cutoff value for the BCG-vaccinated population in the Control of Tuberculosis Guidelines of the Ministry of Health of Turkey and the suggestions of the Centers for Disease, Control and Prevention (11,18).

Blood collection procedures: Venous blood was collected in sodium heparinized tubes prior to intradermal skin testing. Whole blood culture was performed by aliquoting 1 ml of blood in wells of a 24-well tissue culture plate (TPP, Trasadingen, Switzerland) within 2 h of collection. The whole blood was stimulated with either sterile phosphate-buffered saline (nil control antigen, 3 drops), a mitogen (positive control, 3 drops phytohemagglutinin), human PPD (HuPPD, 3 drops), or avian PPD (AvPPD, 3 drops), according to the instructions included in the QFT kit. After 18 h of incubation (37°C, 5% CO2, 95% air, 100% humidity), approximately 250 to 300 μl plasma were collected and stored for later measurement of INF-γ by an enzyme immunoassay (EIA) provided with the QFT kit.

QFT assay: The QFT assay was performed in accordance with the manufacturer’s instructions in 2005. Briefly, 96-well plates coated with an anti-INF-γ monoclonal antibody were purchased. Each well was filled with 50 μl of anti-human INF-γ-horseradish peroxidase conjugate and 50 μl of the test specimen. The plate contents were thoroughly mixed and incubated for 1 h at room temperature. The plates were washed for 6 cycles with 300 μl of washing buffer. A 100-μl portion of substrate was added to each well. The admixture was allowed to develop for 30 min at room temperature, at which time 50 μl of enzyme-stopping solution (1 N H2SO4) was added to halt the reaction. Absorbance was measured at 450 nm using an EIA plate reader (Multiskan EX, Thermo Lab Systems, Helsinki, Finland). The INF-γ in the whole blood supernatant was measured using software provided by the kit manufacturer. M. tuberculosis infection was defined as the percent human response (%HuPPD) ≥15% and the percent avian difference ≥-10% as follows.

%HuPPD = Hu PPD INF-γ - Nil INF-γ ×100

%Avium difference = (AvPPD INF-γ - Nil INF-γ)-(Hu PPD INF-γ - Nil INF-γ) ×100

Statistics: Concordance between the TST and QFT assay was examined by using Cohen’s kappa. Kappa values of <0.4, 0.4 to 0.75, and >0.75 are consistent with poor, good, and excellent concordance, respectively. Categorical data were compared by using the Mantel-Haenszel chi-square test. Continuous variables were tested by using the Student’s t test. A P value less than 0.05 was accepted as statistically significant. All variables showing a significant relationship on the bivariate tests were included in the binary logistic regression model.

RESULTS

The participants consisted of 43 males (56.6%) and 33 females (43.4%). The ages of the participants ranged from 18 to 50 years (mean ± SD, 30.4 ± 5.4). Anti-HIV antibodies were negative in all of the participants by EIA (Johnson & Johnson, Toledo, Spain). The participants had been working in the hospital for 1-20 years (mean ± SD, 3.9 ± 4.7). The QFT results were positive in 65 of 76 HCWs (85.5%). TSTs were positive in 41 of 76 participants (53.9%). All TST-positive individuals had negative chest x-rays and normal physical examinations. They also had normal complete blood counts and liver function tests. There was a significant difference between the numbers of patients with QFT positivity and TST positivity (P = 0.02). TST induration diameters were found to be 9.2 ± 5.9 mm (mean ± SD) in QFT (-) participants and 15.2 ± 5.8 mm (mean ± SD) in QFT (+) participants. The difference between them was significant (P = 0.002). The percentage of patients with QFT positivity increased in accordance with the increase in the TST induration diameter. When the TST induration diameter was ≥20 mm, QFT positivity was 100%. Multivariate analysis revealed that the correlation between QFT positivity and the indura-
tion diameters of TST was significant \( (P = 0.009) \). The factors related to QFT positivity are shown in Table 1, and the relationship between the induration diameters of TST and QFT positivity are shown in Figure 1.

The concordance between TST and QFT was 63.1\% \( (\kappa = 0.219) \), and was not affected by gender or BCG vaccination (Table 2).

### DISCUSSION

HCWs are one of the groups at risk for \( M. \) tuberculosis infection. However, the levels of TB exposure vary widely among the various health care positions. For this reason, early diagnosis of \( M. \) tuberculosis is of critical importance for HCWs. Although the TST is widely used to diagnose TB infection, it does have some drawbacks. The QFT seems to overcome the drawbacks in TST (8).

The Turkish population had the widespread TB infection and the BCG vaccination (11). In the present study, we aimed to compare the TST and QFT results in HCWs of a university hospital in Turkey.

In our study, an increase in the induration diameter by TST was associated with an increase in the number of QFT-positivity patients. Potthumarthy et al. (5) showed that the correlation between the diameter of induration for Mantoux test and the magnitude of the QFT was significant and of moderate strength in HCWs. In contrast, Johnson et al. (13) and Fietta et al. (3) found no correlation between the diameter of TST induration and the magnitude of the QFT response. We did not research the correlation between the magnitude of the QFT response and the diameter of TST induration in our study, but we have shown that the ratio of QFT positivity was increasing while the diameter of TST induration was increasing. The difference between these studies may be due to different immunities in the participants, as well as other factors such as the degree of endemicity for TB, the frequency of BCG vaccination, and the exposure to environmental \( \text{Mycobacterium} \) spp. in the countries examined (8).

Nguyen et al. (19) found that positive QFT in the setting of negative TST frequently anticipates a TST boost. They claimed that this finding helped to explain the discordance between the two tests and may provide an alternative to serial TST testing (19). Kraut et al. (7) reported that some positive TST results in such HCWs were related to non-

### Table 1. The factors related with QFT positivity

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>QFT ( n = 65 (%) )</th>
<th>Logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td>Coefficient</td>
</tr>
<tr>
<td>Male</td>
<td>6 (14.0) 37 (86.0)</td>
<td>0.333</td>
</tr>
<tr>
<td>Female</td>
<td>5 (15.2) 28 (84.8)</td>
<td></td>
</tr>
<tr>
<td>Age (year) mean ± SD</td>
<td>30 ± 4 30 ± 6</td>
<td>0.890</td>
</tr>
<tr>
<td>BCG status</td>
<td>positive</td>
<td>6 (85.7) 59 (85.5)</td>
</tr>
<tr>
<td>Working duration (year) mean ± SD</td>
<td>2.3 ± 2.1 4.3 ± 5.0</td>
<td>0.033</td>
</tr>
<tr>
<td>TB History</td>
<td>No</td>
<td>11 (15.3) 61 (84.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>–</td>
<td>4 (100)</td>
</tr>
<tr>
<td>TST induration diameter (mm) mean ± SD</td>
<td>9.2 ± 5.9 15.2 ± 5.8</td>
<td>0.002</td>
</tr>
</tbody>
</table>

\(^1\) Chi-square test.

\(^2\) Logistic regression analysis.

### Table 2. The concordance between TST and QFT

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>TST ( n = 11 (%) )</th>
<th>QFT (n)</th>
<th>Overall concordance (%)</th>
<th>Kappa statistic (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (76)</td>
<td>Negative</td>
<td>9</td>
<td>26</td>
<td>63.1</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>2</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>BCG – (7)</td>
<td>Negative</td>
<td>1</td>
<td>3</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>–</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>BCG + (69)</td>
<td>Negative</td>
<td>8</td>
<td>26</td>
<td>59.4</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>2</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Female (33)</td>
<td>Negative</td>
<td>3</td>
<td>13</td>
<td>54.5</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Male (43)</td>
<td>Negative</td>
<td>6</td>
<td>16</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>–</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

\(^3\) Negative, <15 mm; positive, ≥15 mm.

\(^4\) A kappa statistic of ≥0.75 represents excellent concordance, 0.40-0.75 represents good to fair concordance, and <0.40 represents poor concordance.
occupational factors, including delayed boosting, rather than to conversion due to recent TB contact.

Tissot et al. (20) determined that the finding of an induration diameter <18 mm by TST may have been related to prior BCG vaccination in a previously vaccinated group under 40 years old. In the light of this finding, it is interesting to note that when the TST induration diameter was ≥20 mm, QFT positivity was 100% in our study. However, the positivity by TST and QFT in vaccinated subjects does not necessarily indicate the presence of LTBI, but merely a conventionally may be defined as “strong hypersensitivity”.

In our study, we found that there was a poor concordance between QFT and TST when the TST reaction was defined as positive with a ≥15 mm diameter induration. It appeared that QFT showed good sensitivity for the diagnosis of LTBI due to both the significant positive correlation between QFT positivity and TST induration diameter and the fact that QFT positivity was observed in all subjects with an induration diameter of over 20 mm by TST. Multivariate analysis revealed that QFT was not affected by BCG vaccination, although the number of BCG-negative cases was small. Mahomed et al. (21) also showed a poor concordance between TST and QFT. Potummarthy et al. (5) showed 68% concordance between QFT and TST in HCWs when a positive TST reaction was defined as ≥15 mm diameter induration. They noted that this concordance was lower than the concordance in groups including TB patients and in immigrants from endemic countries. However, Katial et al. (4) found a good concordance between the TST and QFT when a positive TST reaction was defined as ≥15 mm diameter induration. Pai et al. (22) found a good concordance between TST and QFT in HCWs and determined that BCG vaccination had little impact on the results of either test. The difference in concordance between the TST and QFT in these studies may have been related to differences in the immunology of participants, differences in the BCG vaccination protocols, and to the better sensitivity and specificity of QFT than TST. In addition, while the biological determinants of QFT and TST assays are similar, they are not equivalent (8, 11, 12).

In conclusion, the percentage of the number of patients with QFT positivity was increased in accordance with the TST induration diameter in the present study. Based on the results, QFT thus seems to be more effective for LTBI diagnosis than TST. However, large-scale trials including quantitative measurement of QFT in subgroups formed to account for the division where HCWs are employed and the results of TSTs (e.g., <15 mm, 15 - 20 mm, ≥20 mm) will be needed to confirm the efficacy of QFT in LTBI diagnosis.

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REFERENCES