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COMPARING THE PERFORMANCE OF QUANTIFERON-TB GOLD AND MANTOUX TEST IN DETECTING LATENT TUBERCULOSIS INFECTION AMONG IRANIAN HEALTH CARE WORKERS

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Abstract
Objectives: The risk of transmission of *Mycobacterium tuberculosis* from patients with tuberculosis to health care workers (HCWs) has been well documented but little is known about the prevalence of latent tuberculosis infection (LTBI) in Iranian HCWs. The aim of this study was to determine the prevalence of LTBI among HCWs by using IFNgamma-release assay and compare the results with those of tuberculin skin test (TST). **Methods**: Two hundred HCWs were evaluated with both TST and QuantiFERON-TB Gold In Tube test (QFT-GIT). The obtained data were analyzed by SPSS v.16 Software. **Results**: The participants were 73 males and 127 females with the mean age of 34.36±8.26 years. TST was positive in 105 cases (52.5%) and the QFT results were positive in 17 cases (8.5%). There was poor agreement between the two tests (53%, $\kappa = 0.115$). Induration diameter of TST ≥ 10 mm and working duration ≥ 10 years were independent predictors for positive QFT ($p = 0.004$). **Conclusions**: Due to the fact that BCG vaccination has been administered routinely to all HCWs in Iran, specific tests should be introduced for high risk groups. QFT thus seems to be more effective for LTBI diagnosis than TST among HCWs with BCG immunization history.

Key words:
QuantiFERON-TB Gold, Mantoux test, Latent tuberculosis infection, Health care workers

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INTRODUCTION

The transmission risk of *Mycobacterium tuberculosis* from patients infected with tuberculosis (TB) to the healthcare workers (HCWs) has been well documented [1]. Some studies showed that the prevalence and incidence of latent tuberculosis infection (LTBI) were high among HCWs in low and middle-income countries [2,3]. According to the World Health Organization (WHO) report, Iran is a country with moderate incidence of TB, and Iranian HCWs are considered as one of the high risk groups for *M. tuberculosis* infection [4]. Therefore, the diagnosis of LTBI in this group and making decisions to treat is an essential need.

The tuberculin skin test (TST) is an old, traditional method to diagnose the tuberculosis infection and disease [5]. The test measures the hypersensitivity response to the purified protein derivative (PPD) which contains a complex of antigens found in both *Mycobacterium tuberculosis* and other *Mycobacteria*. These antigens frequently cross–react with those of many non-tuberculosis *Mycobacteria* and leads to a high rate of false positive TST result. On the other hand, TST may yield false–positive results in individuals who have been vaccinated with BCG [6].

Recognition of the essential role of interferon gamma (IFN-gamma) in regulating the cell-mediated immune responses to *M. tuberculosis* infection has led to development of interferon gamma release assays (IGRAs) for detecting the latent tuberculosis infection [7]. The QuantiFERON-TB Gold In Tube test (QFT-GIT) is a commercially available IGRA, which uses Enzyme–Linked ImmunoSorbent Assay (ELISA) in order to measure the production of interferon gamma circulating T cells being produced against specific *M. tuberculosis* antigens in the whole blood. The recent diagnostic tool may be used in contact investigations, evaluation of recent immigrants and serial testing surveillance programs for infection control [8].

Accuracy assessments of TST and IGRAs, in order to diagnose the LTBI were impeded by the lack of confirmatory tests [9]. However, published data show that the sensitivity of QFT-GIT is similar to TST and even higher in patients with active tuberculosis [10–12].

Little is known about the prevalence of LTBI in Iranian HCWs [3] and to our best knowledge, there is no study about using QFT-GIT in order to detect LTBI in this high risk group. The aim of the recent study was to compare the performance of QFT-GIT and TST to detect LTBI among HCWs in a teaching hospital. Probable concordance between both test results and associations with known risk factors were also studied

STUDY POPULATION AND METHODS

The study is an observational analytic cross–sectional study, conducted in Rasoul-e-Akram general hospital in Tehran, Iran, during a one-year period from 2009 to 2010. The study was approved by the Ethics Committee, Tehran University of Medical Sciences (TUMS).

A total of two hundred HCWs, all vaccinated by BCG, were recruited for the study. Written informed consent was obtained from each participant. Individuals with positive history for active tuberculosis, using immunosuppressive drugs, suffering from chronic renal and liver failure and with signs or symptoms suggestive of TB were excluded from the study. Information on the following variables was gathered using a questionnaire: age, gender, job category, service and years in the health profession. History taking and physical examination were performed by two expert physicians.

An intradermal Mantoux test was performed for each participant with five units of Tuberculin PPD [SACHIN 394230 (Surat) India]. Investigators injected 0.1 ml of Tuberculin PPD solution into the forearm. The result of the Mantoux test was evaluated between 48 to 72 hours after the injection. Induration was considered while interpreting the test. The diameter of induration was recorded in millimeters. Positive TST was defined as induration ≥ 10 mm.

For all HCWs, a venipuncture was performed and 3 ml of whole blood were collected. The QFT-GIT test was

performed in the Immunology Department, Faculty of Medicine, TUMS, taking into account the manufacturer's recommendations (Cellestis Ltd., Carnegie, Australia).
Briefly, one ml blood was transferred directly into each of three evacuated blood collection tubes. One tube served as the negative control containing only heparin. The second tube served as the positive control containing the T cell mitogen phytohemaglutinin and the third tube containing the *M. tuberculosis* antigens ESAT–6, CFP–10 and TB7.7. The specimens were centrifuged at 2000–3000 rpm for 15 minutes after being incubated upright at 37°C for 24 hours. The supernatant was frozen at –20°C for further analysis.
The test was considered positive when the corrected TB antigen-stimulated plasma level was ≥ 0.35 IU/ml. Results were calculated using the QFT software.

Statistical Analysis

Statistical analysis was performed using SPSS 16 software. Some statistical tests such as t-test, chi square test and logistic regression were used to test the differences in proportions of categorical variables between the groups. P-values less than 0.05 were considered statistically significant. The agreement between the QFT-GIT and TST was calculated using Kappa test.

RESULTS

Two hundred HCWs were tested for LTBI. The participants consisted of 73 males (36.5%) and 127 females (63.5%). The mean age of the participants was 34.36±8.26 years (23–59). Demographic data and the factors related to TST and QFT-GIT positivity are shown in Table 1.
TST was positive in 105 of 200 participants (52.5%) while the QFT-GIT results were positive in 17(8.5%), negative in 181(90.5%) and indeterminate in 2 (1%). According to the immunologist suggestion, data were analyzed while considering indeterminate QFT-GIT results as negative.
Agreement kappa coefficient between TST and QFT–GIT was slight (p = 0.01, k = 0.115). Of the positive TST group (105 cases), 14 (13.33%) had concomitant positive QFT. Of the 95 cases with negative TST test, 92 (96.6%) had negative QFT while 3(3.4%) had positive QFT results.

Table 1. Demographic data and TST, IFN gamma results of 200 HCWs

Characteristic	Total n (%)	Positive TST	P Value	Positive QFT_GIT	P Value
Gender					
female	127 (63.5)	60 (47.2)	0.05	8 (6.2)	0.057
male	73 (36.5)	45 (61.6)		9 (12.3)	
Age (years)					
20–30	85 (42.5)	45 (52.9)	0.2	4 (4.7)	0.1
31–40	70 (35.0)	32 (45.7)		6 (8.5)	
≥ 41	45 (22.5)	28 (62.2)		7 (15.5)	
Occupation					
physician	54 (27.0)	26 (48.1)	0.9	2 (3.7)	0.6
nurse	62 (31.0)	32 (52.2)		5 (8.0)	
nurse – aid	43 (21.5)	25 (58.1)		5 (11.6)	
cleaners	28 (14.0)	15 (53.6)		4 (14.2)	
administrative staff	13 (6.5)	7 (53.8)		1 (7.6)	

Table 1. Demographic data and TST, IFN gamma results of 200 HCWs — cont.

Characteristic	Total n (%)	Positive TST	P Value	Positive QFT_GIT	P Value
Working Duration (years)					
1–10	139 (69.5)	70(50.4)	> 0.1	7 (5.0)	0.08
11–20	46 (23.0)	23 (50.0)		8 (17.3)	
21–30	15 (7.5)	12 (80.0)		2 (13.3)	
Ward					
medical wards (pulmonary & infectious diseases wards)	105 (52.5)	57 (54.3)	0.5	4 (3.8)	0.02
others	95 (47.5)	48 (50.5)		13 (13.6)	

Table 2. Correlation between TST induration and QFT results

QFT	TST		Total	P Value
	0–9 mm	≥ 10 mm		
Positive	3 (17.64)	14 (82.35)	17	0.01
Negative	92 (50.27)	91 (49.72)	183	

Of the positive QFT group (17 cases), 14(82.4%) had concomitant positive TST and of the negative QFT group (183 cases), 91 (49.72%) had concomitant negative TST. The percentage of patients with QFT positivity increased in accordance with the increase in the TST induration diameter. Multivariate analysis revealed that the correlation between QFT positivity and the induration diameters of the TST was significant (p = 0.011) (Table 2).

Multivariate analysis, using Logistic Regression model showed that positive PPD (B = 1.53, p = 0.02) and working duration longer than 10 years (B = 1.05, p = 0.04) are independent predictors for positive QFT (p = 0.004) (Table 3).

Table 3. Agreement between QFT-GIT and TST

Results (TST/QFT-GIT)	Frequency, n (%)
Positive/Positive	14 (7.0)
Negative/Negative	92 (46.0)
Negative/Positive	3 (1.5)
Positive/Negative	91 (45.5)
Agreement between TST & QFT-GIT	106 (53.0)

DISCUSSION

The results of the recent study showed that the frequency of LTBI among HCWs was 8.5% using QTF-GIT, while the TST showed a 52.5% positive frequency percent among the same population. The difference seems to be attributable to the BCG vaccination history which was positive in all of the participants and also the to non-tuberculosis *Mycobacterial* infection [13]. The prevalence of TB among HCWs was determined by QFT release assay in previous studies, with the lowest prevalence in Denmark (1%) and the highest in Turkey (85.5%) [14–23].

The recent study's results are consistent with other reports, concluding that increase in age and work history in health care system are associated with higher prevalence of LTBI [15,18,20,23–24], although, Rifaza et al. found no association between duration of employment and risk of LTBI [21].

On the other hand, the prevalence of LTBI in HCWs in the clinical setting was not significantly different from

those working in the non-clinical environment [21,22]; these findings somewhat contradict the results of other studies in which physicians and nurses showed a higher prevalence rate than the administrative staff [2,15,18].

There are several reports about rates of TB by work location. While Joshi et al. calculated an incidence rate ratio (IRRs) for the workers in TB inpatient facilities (14.6–99.0), laboratories (78.6), general medicine wards (3.9–36.6) and emergency rooms (26.6–31.9), who had a higher risk for TB infection compared with the general population [2] some other authors showed no significant relationship between the occurrence of TB and the work location [15,18,21,25]. Our study showed that working in ICU, surgery ward and emergency department was significantly associated with higher LTBI. It is assumed that the increased risk of TB disease among Iranian HCWs is attributable to the failure to use personal protective gear.

Our findings support those of other studies, which also showed a correlation between the TST induration diameter and QFT-IGT positivity [14,18]. Pottumarthy et al. revealed that the correlation between the diameter of induration for TST and the magnitude of the QFT was significant [26]. In contrast, Fietta et al. showed that in subjects with active TB or those vaccinated with BCG, the agreement between assays was poor and no correlation was found between the diameter of TST induration and the magnitude of the IFN-gamma responses. They concluded that QFT cannot be considered as an appropriate alternative for the TST in screening for latent infections [11]. The difference between these studies may be due to the degree of endemicity for TB, the frequency of BCG vaccination, and the exposure to *Mycobacterium tuberculosis* in the regions examined [17]. In our study, the observed poor agreement (53%) between the TST and QFT-GIT results could be explained by BCG vaccination.

Nienhaus et al. found TST-positive/QFT-negative result was related to BCG vaccination [27]. In a population of healthcare staff with a low prevalence of TB and a significant rate of BCG vaccination, positive QFT was associated with the presence of known risk factors for TB exposure, whereas a positive TST result was more strongly associated with a prior history of BCG vaccination [18,28]. BCG vaccination is the main problem in interpreting TST results [16], particularly in countries with high rate of vaccination. The study performed by Tissot et al. about BCG influence on TST results concluded that TST reactions of ≤ 18 mm in individuals < 40 years of age with positive history for BCG vaccination should be interpreted with caution [29]. Casas et al. showed that the agreement between TST and IFN-gamma test results in non-BCG vaccinated HCWs was higher compared to BCG vaccinated cases [17]. In contrast, Pai et al. found a good agreement between QFT and TST in HCWs and claimed that BCG vaccination had little impact on the results of either test [20].

In our experience, we have detected three HCWs with a positive QFT test and negative TST results. These results could be interpreted as a false-positive QFT test, or its greater sensitivity to detect LTBI. Indeed, this kind of discrepancy has been previously found by other authors [17,30–31], and is not explained properly. More further detailed studies seem to be necessary.

We followed our cases for six months and none of them except one (TST+/QFT–), showed signs of active tuberculosis. There are conflicting data about the potential risk of progress towards active TB in TST+/QFT– cases and TST–/QFT+ individuals [32–33]. However, based on Nienhaus study, it is likely that the chance of cases with TST+/QFT– results to progress toward TB is small; we need more time to follow our cases to assess this chance exactly [27].

Due to the fact that BCG vaccination has been administered routinely in neonates in Iran, specific test should be introduced in high risk groups.

Tuberculosis National Committee in Iran should decide which approach is more practical to define LTBI; TST followed by IGRA or the traditional TST followed by Chest

X-Ray and clinical follow up of the positive cases. Wrighton-Smith et al. showed that the second approach seems to be more expensive than the first one [34].

Despite the considerable specificity for new IGRAs, due to the fundamental limitation of lack of the gold standard of diagnosis of LTBI, additional studies are needed to assess the performance of IGRA while comparing it with the TST in high risk population [35].

Despite all these limitations, our work was one of the few studies among HCWs to detect LTBI by using QFT-GIT test and could be a base for further large-scale trials [15,21].

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