

Screening of latent tuberculosis infection among health care workers working in Hajj pilgrimage area in Saudi Arabia, using interferon gamma release assay and tuberculin skin test

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BACKGROUND: Interferon gamma release assays (IGRA) is highly specific for *Mycobacterium tuberculosis* and is the preferred test in BCG-vaccinated individuals. The few studies that have screened health care workers (HCWs) in Saudi Arabia for latent tuberculosis infection (LTBI) using IGRA have varied in agreement with the traditional tuberculin skin test (TST).

OBJECTIVE: Assess the prevalence of LTBI among HCWs working in the Hajj pilgrimage using IGRA and TST and measuring their agreement.

DESIGN: Cross-sectional prospective.

SETTING: Multiple non-tertiary care hospitals.

PATIENTS AND METHODS: HCWs who worked during the Hajj pilgrimage in Saudi Arabia in December 2015. Data was collected by standardized questionnaire. Samples were drawn and analyzed by standard methods.

MAIN OUTCOME MEASURES: The prevalence of LTBI among HCW and the agreement by kappa statistic between QFT-GIT and TST.

SAMPLE SIZE: 520 subjects.

RESULTS: The sample included 30.7% nurses and 19.2% physicians. The majority were BCG vaccinated (98.5%). There were a total of 56 positive by QFT-GIT and the LTBI rate was 10.8%. In 50 QFT positive/476 TST negative the LTBI rate was 10.5% in discordant tests, and in 6 QFT positive/44 TST positive it was 13.6% in concordant tests. The overall agreement between both tests was poor—83% and kappa was 0.02. LTBI prevalence was associated with longer employment (13.1 [9.2] years). The QFT-GIT positive test was significantly higher in physicians ($P=.02$) and in HCWs working in chest hospitals 16/76 (21.05%) ($P=.001$).

CONCLUSION: Agreement between the tests was poor. QFT-GIT detected LTBI when TST was negative in HCWs who had a history of close contact with TB patients.

LIMITATIONS: A second step TST was not feasible within 2-3 weeks.

CONFLICT OF INTEREST: None.

Interferon gamma release assays (IGRA), particularly the Quantiferon Gold in Tube test (QFT-GIT), are the preferred diagnostic tools for screening of latent tuberculosis infection (LTBI) in Bacillus Calmette-Guerin (BCG)-vaccinated individuals.^{1,2} The use of the QFT-GIT test alone is still not recommended in the screening of LTBI because the association between IGRA when compared to the tuberculin skin test (TST) among health care workers (HCWs) in tertiary care hospitals has variable and differing agreement.^{3,4} Several systematic reviews have suggested that IGRAs are as sensitive and more specific than TST in identifying high-risk people for TB infection particularly in low incidence areas.⁵⁻⁸ The sensitivity of QFT has been consistently reported as 81% to 86%, equal to the sensitivity of the TST (71%–82%). However, the specificity of QFT has been high (94–98%) compared to a low TST specificity (47–70%) in BCG-vaccinated individuals.^{1,9} TB in Saudi Arabia has a moderate incidence rate of 10 per 100 000 population according to World Health Organization (WHO) reports in 2017.⁹ Close contacts and mass-gathering from all over the world during Hajj pilgrimage can potentially transmit airborne diseases such as *Mycobacterium tuberculosis* (MTB) infection to HCWs. Annual screening of LTBI using TST traditionally can detect TB infection among HCWs with high occupational risk, especially those who provide care during Hajj pilgrimage.^{10,11} The use of IGRAs for routine screening of HCWs remains an area of controversy and many international guidelines (Europe and Canada) have not endorsed IGRAs alone for testing in a healthcare setting.^{2,12-18}

IGRA is a single blood test that measures the T-cell-mediated immune response and the interferon (IFN)-gamma release following incubation with MTB-specific antigens. There are two commercial QFT tests (QFT-GIT and TB spot). The QFT-GIT assay is an enzyme-linked immunosorbent assay (ELISA)-based, whole-blood test that uses peptides from three TB antigens (ESAT-6, CFP-10, and TB7.7) in an in-tube format. The result is reported as quantification of interferon (IFN)-gamma in international units (IU) per mL. The TB spot test is preferable in immunocompromised patients.^{1,19,20} IGRA advantages include convenient or favorable fast results with no follow-up visit required to complete the testing process, especially for the busy HCWs. There are no false-positive results in prior BCG-vaccinated individuals or those who are previously sensitized with non-tuberculous mycobacterium (NTM). IGRAs overcome the challenges related to TST for the screening of LTBI such as the use of injection, inconvenient return in 48–72 hours for reading of results, the process of training HCWs for proper test performance and the inter-reader

variability in interpretation. TST is a delayed-type hypersensitivity response to the purified protein derivative (PPD) of MTB, with false positive results in individuals who are previously BCG vaccinated or with NTM infection, and has a low sensitivity for active TB.²¹⁻²³

Local Saudi guidelines on the management of latent tuberculosis do not recommend using IGRAs for the diagnosis of active tuberculosis.²⁴ The positive predictive value of IGRA is low because it does not differentiate between active TB disease or LTBI. As it is controversial, authors think clinical action is necessary following the positive IGRAs to rule out active TB disease. Other limitations include the reproducibility of results in different laboratories, blood collection and samples processing.²⁵⁻²⁹ Our objective was to assess the prevalence of LTBI among HCWs working in Hajj pilgrimage using QFT-GIT test and TST and to measure the association or agreement of the two tests.

PATIENTS AND METHODS

Any physician, nurse or other HCW who did not have a positive TST and who worked in the Hajj pilgrimage during their employment were invited to participate voluntarily in the study. The enrollment period began after the Hajj and last for three months. Inclusion criteria required that participants be HCWs who previously worked in the Hajj pilgrimage, 22 years of age or older, employed for more than two years, and not have had close contact with pulmonary TB patients within the previous three months. Exclusion criteria included pregnancy, immunoincompetency, employment as administrative staff, contact with a pulmonary TB patient within the previous three months, or a recent history of a positive TST. The research was approved by the Deanship of Research in Taibah University#3104/435 and approved by the IRB and deanship of research through the scientific ethical committee.

A data collection questionnaire designed by the investigators was used to collect information by face-to-face interview on gender, age, nationality, workplace, job classification, duration of employment in hospitals, BCG vaccination, history of contact with TB patients and history of associated co-morbidity. Data were collected by trained investigators and entered into the computer for statistical analysis by different investigators. An investigator blinded to the data collection or interviews did the data analysis. The clinical evaluation was done in the clinic by separate physician investigators. Investigators were supervised by the primary investigator at the beginning of data collection to ensure that instructions and questions were clear to investigators and participants.

Blood volumes were collected by a trained HCW after informed consent was obtained from all participants and just before TST performance. Three mL of whole blood was collected from each participant. For the QuantiFERON-TB Gold In-Tube test (QFT-GIT), 1 mL delivered into each of the three tubes labeled as nil control, positive control and *M tuberculosis* specific antigens (ESAT-6 and CFP10). The blood was incubated without delay, with the test antigens ≤ 12 hours after collection. Tubes were incubated at 37°C overnight for 16–24 before centrifugation. QFT-GIT kits (Cellestis Limited, Carnegie, Australia) contain three types of tubes, the *M tuberculosis* specific antigens tubes which include two mixtures of synthetic peptides representing ESAT-6 and CFP-10 as test antigens, phytohemagglutinin (a mitogen used as a positive assay control), and saline (used as a nil sample to measure the background level of IFN-g).

After incubation, the concentration of IFN-gamma in the plasma is determined by ELISA, using the reagents included in the test kit and the PEB III (Dade Behring). The amount of IFN-gamma released is determined by subtracting the amount in the nil tube from the amount in the ESAT-6, CFP-10, or mitogen-stimulated plasma.

Interpretation Criteria for QFT-GIT			
Interpretation	TB Specific Antigen Response (IU/mL)*	Nil Control (IU/mL)	Mitogen Control (IU/mL)*
Positive	≥ 0.35 (and $\geq 25\%$ of Nil)	≤ 8.0	any
Negative	< 0.35 (or $< 25\%$ of Nil)	≤ 8.0	≥ 0.5
Indeterminate	< 0.35 (or $< 25\%$ of Nil)	≤ 8.0	< 0.5
	any	> 8.0	any

*Corrected for nil response.³⁰ A summary of meeting proceedings on addressing variability around the cut point in serial interferon- γ release assay testing. Infect Control Hosp Epidemiol 2013; 34:625-630.

QFT-GIT results were calculated quantitatively, using the software in the manufacturer guidelines to interpret test results, as shown in the interpretation criteria table. The QFT was considered positive or negative based on the IFN-concentration cut off value of 0.35 IU/mL, using the interpretation criteria in the interpretation criteria table.³⁰

The anticipated practical limitations of QFT-GIT were avoided and all samples were incubated less than

12 hrs after collection in a standard single laboratory. A history of BCG was determined and all participants who had positive TST or positive QFT-GIT test were assessed with recent chest X-ray reviewed with the infectious diseases specialist to rule out active TB diseases. Blood sampling was done just prior to TST. An individual is considered positive for *M. tuberculosis* infection if the IFN-gamma response to TB antigens is above the test cut-off (after subtracting the background IFN-gamma response in the negative control). TST uses a single step 0.1 mL (2 TU) of purified protein derivative (RT23; Copenhagen, Denmark) intradermal injection on the volar side of the forearm, and the transverse diameter of the induration was read 48 to 72 h later. An induration of 10-mm diameter or more was considered positive. Challenges included lab transportation without delay, and the concern of some HCW over accidental venipuncture.

The data were analyzed using statistical analysis software package SPSS version 16 (<https://www.ibm.com/products/spss-statistics>). The authors were blinded to results. Data is presented as frequencies, mean and standard deviation as appropriate. The Chi-square and the Fisher exact test were used to test the significance between the qualitative variables. The independent t test used to test the significance between the quantitative variables. *P* value $\leq .05$ was considered of statistically significant. The agreement between QFT-GIT and TST was assessed by kappa where $\kappa > 0.75$ represent excellent agreement, κ values from 0.4–0.75 represent fair to good agreement and $\kappa < 0.4$ represent poor agreement.^{30,31}

RESULTS

The study included 520 HCWs from Saudi Arabia working at multiple secondary care hospitals in the Hajj pilgrimage region. The mean (SD) age was 34.9 (9.7) years with the youngest 22-years-old and the eldest of 62 years old. There were 66.9% males, and 33.1% females. The majority were non-Saudi, mainly from Egypt (17.1%) Philippines (15.2%), Bangladesh (12.3%), Sudan (3.2%) and others from Yemen, Syria, and India (9.6%) (Table 1). Of the 520 HCWs enrolled 50% were clinical staff involved with direct patient care. The mean (SD) length of employment was 10.3 (9.3) years. Most worked in secondary care non-chest hospitals. The majority of studied population had a history of BCG vaccination and almost half had had close contact with TB patients. Only 8.5% had a positive TST, and all had a normal chest X-ray (CXR).

A total of 56/520 (10.8%) had a positive QFT-GIT (Table 2). All HCWs with a positive TST or QFT-GIT

were assessed with CXR, and the data collected for the study. The clinical well-being and normal radiology was documented for the diagnosis of LTBI in this group of HCWs. The mean age was significantly higher in QFT-GIT positive participants compared to QFT-GIT negative participants ($P=.01$). There was no statistically significant differences in QFT-GIT test results among Saudis versus non-Saudis. (Table 2). QFT-GIT positive was significantly higher in physicians compared with other job classifications ($P=.02$). The QFT-GIT positive test was higher in HCWs working at chest hospitals than HCWs in other hospitals ($P=.001$). The duration of employment was significantly longer in QFT-GIT positive participants compared to the others ($P=.02$). There was no difference in close contact with TB patients. There was also no significant associated comorbidity, such as diabetes or chronic steroid use, among the QFT-GIT HCWs. The majority of HCWs with positive QFT-GIT results had a negative TST. Concordant results were obtained in 13.6% of TST positive/QFT positive cases and 89.5% of TST negative /QFT negative cases (Table 2). TST positive/QFT negative discordance was 86.4% while TST negative/QFT positive discordance was 10.5%. The overall agreement between results of two tests was 83%, with a kappa 0.02 which is considered poor agreement ($\kappa < 0.4$) (Table 3).

DISCUSSION

Respiratory infections and communicable diseases are challenging hazards to HCWs, especially with the close contact that occurs during mass gathering like the Hajj pilgrimage.^{32,33} There are few studies in Saudi Arabia with variable agreement using both (QFT-GIT and TST) as risk assessment methods for TB infection. The agreement between (QFT-GIT and TST) in the pre-employment screening for HCWs, and in post exposure screening has been reported as fair to good.^{3,4} These studies were implemented on geographically diverse study populations at tertiary care hospitals. The association or agreement between both methods (QFT-GIT and TST) was different in other studies, with poor agreement in renal dialysis, and renal transplant patients.³⁴⁻³⁶ The HCWs working in Hajj pilgrimage area with LTBI in our study (10.5%) had discordant results for QFT-GIT positive/TST negative (50/476). There were 6 (13.6%) HCWs with concordant QFT-GIT positive/TST positive (6/44). In two different studies in Saudi Arabia, the prevalence of LTBI using both QFT and TST was 31.5% in 2012-2015 compared to 11% using TST alone in 2010.^{4,37}

The overall poor agreement between both tests is consistent with a tertiary care study in Saudi Arabia in

Table 1. Demographic and clinical characteristics of the health care workers (n=520).

Characteristics	Number (percent) or mean (SD)	
Sex		
Males	348	66.9
Females	172	33.1
Age (range) (years)	34.9 (9.7), 22-62	
Nationality		
Saudi	221	42.5
Non-Saudi	299	57.5
Job classification		
Physician	100	19.2
Nurse	160	30.8
Pharmacist	70	13.5
Lab. Technicians	50	9.6
Housekeeper	70	13.5
Radiology Technicians	70	13.5
Duration of employment (years)	10.3 (9.3)	
Diabetes mellitus type 2		
Yes	24	4.6
No	496	95.4
Use of steroid		
Yes	4	0.8
No	516	99.2
BCG vaccine		
Yes	512	98.5
No	8	1.5
Contact with TB patient in the past (more than 12 weeks prior to participation in the study)		
Yes	200	38.5
No	320	61.5
Type of hospital place of work		
Chest hospital (Pilgrims hospital)	76	14.6
Multicenter non chest hospitals-secondary care	444	85.4
Tuberculin skin test		
Positive (more than or equal to 10 mm induration)	44	8.5
Negative (less than 5 mm induration)	476	91.6

Data are mean (standard deviation) or number (percentage).

Table 2. Factors tested for significant association with positive QFT-GIT results.

Variable	Positive QFT-G N=56 (10.77%)		Negative QFT-G N= 464 (89.23%)		Total	P value
	NO.	%	NO.	%		
Mean age in years (SD)	38.0 (10.3)		34.5 (9.6)			.01
Sex						
Males	46	13.2	302	86.8	348	.01
Females	10	5.8	162	94.2	172	
Nationality						
Saudi	28	12.7	193	87.33	221	.25
Non Saudi	28	9.3	271	90.7	229	
Job classification						
Physician	20	20.0	80	80.0	100	.02
Nurse	12	7.5	148	92.5	160	
Pharmacist	4	5.7	66	94.3	70	
Lab. Technicians	4	8.0	46	92.0	50	
Housekeeper	10	14.3	60	95.0	70	
Radiology technicians	6	8.7	64	85.7	70	
Duration of employment in years	13.1 (9.8)		10.0 (9.1)			.02
Diabetes mellitus type 2						
Yes	4	16.7	20	83.3	24	.34
No	52	10.5	444	89.5	496	
Use of steroid						
Yes	0	0.0	4	100.0	4	.69
No	56	10.9	460	89.1	516	
BCG vaccine						
Yes	56	10.9	456	89.0	512	.32
No	0	0.0	8	100.0	8	
Contact with TB patient						
Yes	22	11.0	178	89.0	200	.89
No	34	10.6	286	89.4	320	
Type of hospital						
Chest hospital (Pilgrims hospital)	16	21.1	60	79.0	76	.001
Multi center non chest hospitals	40	9	404	91.0	444	
Tuberculin skin test						
Positive	6	13.6	38	86.4	44	.609
Negative	50	10.5	426	89.5	476	

Data are mean (standard deviation) or number (percentage)

Table 3. Agreement between QFT-GIT and tuberculin skin test in the studied groups.

Group	Overall agreement	kappa	P value
All	83%	0.02	.52

2017 by Edathodu et al, who evaluated the diagnostic potential of interferon-gamma release assay to detect latent tuberculosis infection in kidney transplant recipients with similar poor agreement.³⁶

To our knowledge, our study is the second largest following another large one.⁴ Our study was a cross-sectional, prospective screening of LTBI in HCWs in Saudi Arabia using IGRA and TST, which measured the association and assessed the poor agreement between both QFT-GIT and TST. We found similar numbers of QFT positive HCWs among Saudis and non-Saudis, indicating little apparent difference among nationalities. Occupational hazards such as being a HCW, longer employment, and working in areas of mass gathering were associated with higher prevalence of LTBI, which was mainly diagnosed using QFT-GIT regardless of the nationality or the geographical origin of HCWs. The pattern of discordant positive QFT-GIT/negative TST was significantly affected with the job category, with physicians differing from others. Working in a chest hospital was associated as a major risk of TB exposure and LTBI compared to non-chest hospitals. Subjects with LTBI had higher mean duration of employment. Based on the annual risk of TB in the studied population, TB guidelines and TB program, all HCWs have a moderate probability of TB transmission, considered to be high in chest hospitals.⁹

Physicians in this study were fewer in number than nurses (100/160) when compared to the literature, where nurses were found to be the profession associated with the most concordant results and physicians were the least with discordant positive QFT-GIT/negative TST.⁴ However, in El Helaly et al retrospective pre-employment screening of HCWs job type or being with clinical vs non-clinical profession was not associated with concordant results of both tests.³

Findings in this study involving HCWs with medium-to-high occupational risk, and who were in majority BCG vaccinated can be explained by the the

biologic limitations and the waning immunity of BCG routinely given immunization at birth or in infancy or early childhood;^{38,39} the negative TST is unlikely to be due to technical limitations, IGRA being approved in the literature with specificity of 98.1% and a sensitivity of 89.0%;⁴⁰⁻⁴² IGRAs not affected by BCG vaccination status and when 13.63 % of the study population had concordant positive QFT-GIT with TST positive they were more useful for evaluation of LTBI.⁴³

The strengths of this study were that screening using QFT-GIT enabled the detection of 10.5% of positive HCWs with LTBI. In addition, blood sampling for QFT-GIT was done just before the TST, so QFT-GIT was an alternative method to detect LTBI when TST was negative. No intermediate results were reported for QFT-GIT. Our investigators were carefully trained for performing the TST. The IGRA results were measured quantitatively by the lab and reported qualitatively as positive/negative. The interpretation of IGRA results was carried out according to the clinical profile and radiologic assessment of each participant among HCW, and consequently the analysis of results were more thorough and required longer time. Our subjects were studied prospectively and are the most recent representative sample when compared to previous studies in Saudi Arabia, where subjects were enrolled either retrospectively, or before the date of this study.^{3,35,36,37} The window period required for QFT to be positive post-exposure was carefully considered when subjects were enrolled in the study since TB exposure within 3 months was the criteria for enrollment. Our results reflect LTBI with little likelihood of false positive and false negative with IGRA specificity of 98.1% and a sensitivity of 89.0%.⁴¹⁻⁴³ All TST negative results were based on less than 5 mm of induration, and all positive greater than or equal to 10 mm. There were no intermediate positive results from 5 to 9 mm. A limitation of the study is that the second step TST in HCWs was not feasible within 2-3 weeks when TST was negative.

In conclusion, the prevalence of LTBI in HCWs using QFT-GIT was 10.5% and was higher in physicians with longer duration of employment and working in chest hospitals. The QFT-GIT and TST results were in poor agreement. QFT-GIT positive detected LTBI when TST was negative in HCWs who had a history of close contact with TB patients.

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