Original Article

Comparison of tuberculin skin test and QuantiFERON-TB Gold In-Tube test in Bacillus Calmette–Guerin-vaccinated children

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ABSTRACT

Objectives: The aim of this study is to determine the concordance between QuantiFERON-TB Gold In-Tube (QFT-GIT) and tuberculin skin test (TST) in children vaccinated with Bacillus Calmette–Guerin (BCG). **Methods:** This cross-sectional study was done at a pediatric tertiary care center in 33 BCG-vaccinated children aged 6 months–15 years suspected of *Mycobacterium tuberculosis* infection or in contact with a patient with open tuberculosis (TB). All patients were tested for TST with purified protein derivative-S 5 tuberculin units and QFT-GIT assays. Concordance was evaluated between TST and QFT assay by kappa coefficient (k). Agreement between the tests was classified into categories: poor if $k < 0.20$, fair ($k = 0.21$–0.40), moderate ($k = 0.41$–0.60), good ($k = 0.61$–0.80), and very good ($k = 0.81$–1.00). **Results:** Both the TST and QFT assay were positive in 13 and negative in eight children, respectively, resulting in an agreement of 63% ($\kappa = 0.31$). Eight children were <4 years of age of which only one patient had a positive TST and QFT-GIT, and TST and QFT-GIT were negative in two patients resulting in an agreement of 37.5% ($\kappa = 0.063$). Among children 4 years of age and older, 12 patients had a positive TST and QFT-GIT and 6 patients had a negative TST and QFT-GIT resulting in an agreement of 72% ($\kappa = 0.41$). Among 12 children who had been in contact with an adult having open TB, both the TST and QFT-GIT were positive in 6 patients and negative in two patients, respectively, resulting in an agreement of 66% ($\kappa = 0.41$). TST specificity was only 29.6% with a positive predictive value of 42.4% as compared to QFT-GIT. Among children <4 years of age, TST specificity was only 28.6% with a positive predictive rate of 16.7%, and among children >4 years of age, TST specificity was 50% with a positive predictive value of 66.7%. In patients with contact with a patient having TB, TST specificity was 33.3%. Considering TST of 15 mm and above as positive, TST specificity increased to 63.2% and a positive predictive value was 56.3%. **Conclusion:** The concordance of TST and QFT-GIT is low in children with previous BCG vaccination and especially in children <4 years of age. QFT-GIT may help to rule out false-positive TST.

KEY WORDS: Children, Mantoux test, QuantiFERON, tuberculin skin test

INTRODUCTION

One-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (MTB), resulting in so-called latent tuberculosis infection (LTBI).[1,2]

Although persons infected with MTB do not manifest overt symptoms of active tuberculosis (TB) and are not infectious, they are at increased risk for developing

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disease and becoming infectious. The rate of progression is faster in children. LTBI itself progresses to active disease in approximately 5%–10% of infected persons.[3] The estimated 2 billion people living with LTBI represent a vast reservoir of potential cases of TB around the world.[4] This reservoir of LTBI is, therefore, a major barrier to the ultimate control and elimination of TB. The diagnosis of LTBI is difficult, and there is no gold standard test available. During the past century, tuberculin skin test (TST) was the only available diagnostic tool for detection of infection with MTB. A positive TST result would reflect MTB infection, a past Bacillus Calmette–Guerin (BCG) vaccination, or environmental *Mycobacterium* exposure.[5] After 2001, the recognition that interferon-gamma (IFN-γ) plays a critical role in regulating cell-mediated immune responses to MTB infection led to the development of *in vitro* Interferon-Gamma Release Assays (IGRAs) for detection of MTB infection.[6] In such persons, sensitized memory/effector T-cells produce IFN-γ in response to these MTB antigens, allowing a biologic basis for IGRAs.[2] Research over the past decade has resulted in the development of two commercial IGRAs. The QuantiFERON-TB Gold (QFT-G, Cellestis, Australia) and the newer version QFT Gold In-Tube (QFT-GIT, Cellestis, Australia) are whole-blood based enzyme-linked immunosorbent assays (ELISA) measuring the amount of IFN-γ produced in response to specific *M. tuberculosis* (antigens used in QFT-GIT are Early Secretory Antigenic Target-6 (ESAT-6), Culture Filtrate Protein-10 (CFP-10) and TB7.7 while those used in QFT-G are ESAT-6 and CFP-10). The MTB antigens used in IGRAs are absent from BCG and most of the nontuberculous mycobacteria, thus decreasing incidence of false-positive results due to these infections or previous BCG exposure as compared to TST. However, ESAT-6 and CFP-10 are present in *Mycobacterium kansasii, Mycobacterium szulgai,* and *Mycobacterium marinum*, and sensitization to these organisms might contribute to the release of IFN-γ in response to these antigens and cause false-positive IGRA results.[3] In tested populations of persons unlikely to have *M. tuberculosis* infection, pooled QFT-GIT specificity was 99%[7-10] and pooled TST specificity from these cohorts was 85%.[8,10] In studies that compared the sensitivity of QFT-GIT to that of TST in patients with active TB, there have been variable results with no statistically significant difference between the two tests,[8-13] greater sensitivity for TST,[14-16] and greater sensitivity for QFT-GIT, respectively.[17,18] Studies in children have suggested that young age may have a negative effect on the performance of IGRAs, mainly because of frequent indeterminate results due to lower IFN-γ production in response to the mitogen control.[19-22] Furthermore, several studies show that QFT-G/QFT-GIT to be more specific than the TST for MTB detection in children.[19,23-26] In India, BCG vaccine is given to all children at birth as part of the universal immunization program. We undertook this study to determine the concordance between QFT-GIT and TST in children vaccinated with BCG.

MATERIALS AND METHODS

Setting and study design
This cross-sectional study was conducted in the pediatric TB clinic at a tertiary pediatric hospital in Mumbai from December 2011 to May 2012 after approval from the institutional ethics committee. All children aged 6 months–15 years who had received BCG vaccine and suspected to have MTB infection or in contact with patients with open TB and referred to the pediatric TB clinic were included in the study. A total of 184 patients were referred to the pediatric TB clinic during the study period; however, 151 patients were already on treatment with antituberculous therapy (ATT) and were excluded from the study. Thus, 33 patients were enrolled in the study.

Patient demographics and data management
Details of each patient were recorded in a clinical pro forma by the investigator, and features such as clinical features, malnutrition (weight or height <5th centile as per the Agrawal's chart),[27] prior treatment for TB, BCG scar, and contact with active TB cases were noted in each patient. Investigations such as chest X-ray, ultrasound abdomen, and tissue culture and histopathology were done as and when required. Both the tests (TST and QFT-GIT) were performed on all the children. Patients were categorized into three groups, namely active TB, LTBI, and no TB. Patients were considered to have LTBI if they were in contact with a patient having open TB, were asymptomatic with normal chest X-ray, and had both a positive TST and QFT-GIT. Patients were considered to have active TB if they had signs and symptoms suggestive of TB and diagnostic workup yielded the diagnosis of TB, and finally, ATT was started. Patients were determined to have no TB if diagnostic workup excluded the disease.

Exclusion criteria
Patients with HIV disease, children with congenital immunodeficiencies, and those being treated with immunosuppressants were excluded from the study. Furthermore, patients who had received ATT for >3 days at the time of referral were also excluded from the study. Patients who had received TST by 10 tuberculin units (TU) were excluded from the study.

Tuberculin skin testing
All TSTs were performed with purified protein derivative (PPD)-S 5 TU produced by SPAN diagnostics. TST was given by the Mantoux test and was administered intradermally on the volar aspect of the forearm by a single trained staff nurse. The TST induration was checked by a single trained resident doctor or nurse after 48 h. Both the staff nurse and resident doctor were without access to QFT-GIT results. An induration of at least 10 mm was considered positive irrespective of BCG status, in tune with the standard practice in India.[28]

QuantiFERON-TB Gold In-Tube assay

All QFT-GIT assays were performed at a single internationally recognized laboratory by a single immunologist. All the samples were processed within 3 h of its extraction. The QFT assay was performed as per the manufacturer's instructions.[29] The assay involved two stages: the first stage involved incubation of whole blood with antigens and the second stage involved 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 a negative control, another also contained mitogen as a positive control, and the third tube had overlapping peptides representing the entire sequences of ESAT-6 and CFP-10 and another peptide from a portion of the TB antigen TB7.7. Within 2–6 h of blood draw, the tubes were incubated at 37°C. After exactly 24 h of incubation, the tubes were centrifuged and plasma was tested for IFN-γ response by ELISA. IFN-γ values (IU/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, the cutoff value for a positive test was IFN-γ ≥0.35 IU/ml. A QFT reading <0.35 IU/ml was considered negative and <0.35 IU/ml with a mitogen response of <0.5 was considered indeterminate.[28]

Reading of results from the two tests was blind, the laboratory being without access to clinical data and the doctor and nurse performing the TST not knowing the results of the QFT results before TST interpretation.

Statistical analysis

Collected data from the clinical pro forma and the results of TST and QFT-GIT were entered into SPSS, version 16 (SPSS 16.0, IBM, Armonk, NY, USA). The data description is expressed in absolute frequencies, using mean and standard deviation or median and range. Concordance was evaluated between TST and QFT assay using two indices: proportion agreement and kappa (κ) coefficients. Risk factors evaluated included age and history of contact with infectious TB (contact was defined as a person staying in the same house or a caregiver having sputum smear or culture-positive TB in the past 2 years). We calculated the specificity of TST considering QFT-GIT as the standard reference. Furthermore, considering TST of 15 mm and above as positive in BCG-vaccinated children, concordance for TST and QFT assay was also calculated. Agreement between the tests were classified into categories: kappa coefficient – poor if $k < 0.20$), fair ($k = 0.21$–0.40), moderate ($k = 0.41$–0.60), good ($k = 0.61$–0.80), and very good ($k = 0.81$–1.00).

RESULTS

A total number of 33 patients were enrolled in the study. The baseline characteristics of patients enrolled in the study are depicted in Table 1.

Table 1: Baseline characteristics of patients enrolled in the study

Clinical features	Mean±SD	n (%)
Age (years), range	5.9±3.2 (1 year-11 years)	
Male		18 (54.5)
Female		15 (45.5)
Contact with TB		12 (36.4)
Past TB treatment (years ago), range	1.2±0.7 (4 months-2 years)	5 (15.2)
Malnourished		14 (42.4)
Fever (days)	34.9±16.5	18 (54.5)
Cough (days)	39.6±47.1	15 (45.5)
Loss of weight		11 (33.3)
Loss of appetite		15 (45.5)
Abdominal pain		9 (27.3)
Pleural effusion		2 (6.1)
Lymph nodes		18 (54.5)
Abnormal chest X-ray		11 (33.3)
Previous TST (years ago), range	1-3 years	3 (9.1)
Diagnosis		
Not TB	19	19 (57.5)
Active TB	8	8 (24.2)
LTBI	6	6 (18.2)

SD: Standard deviation, TST: Tuberculin skin test, TB: Tuberculosis, LTBI: Latent TB infection

Tuberculin skin test and QuantiFERON-TB Gold In-Tube results

Out of 33 patients, 24 (72.7%) had a positive TST result with a mean induration of 17.8 ± 5.2 mm. Fourteen (42.4%) patients had a positive QFT-GIT result with a mean reading of 2.9 ± 2.5 IU/ml with a range of 0.48–7.54 IU/ml while 19 (57.5%) had a negative QFT-GIT with a mean reading of 0.05 ± 0.07 IU/ml. No patient had an indeterminate QFT-GIT result. The average QFT-GIT response in patients with active TB was 2.8 IU/ml and those with LTBI was 2.98 IU/ml and in those with no TB was 0.05 IU/ml.

Agreement between tuberculin skin test and QuantiFERON-TB Gold In-Tube assay results

Both the TST and QFT assay were positive in 13 and negative in 8 children, respectively, resulting in an agreement of 63% ($\kappa = 0.31$). Eight children were <4 years of age and only one patient had a positive TST and QFT-GIT, and TST and QFT-GIT were negative in two patients resulting in an agreement of 37.5% ($\kappa = 0.063$). Among children 4 years of age and older, 12 patients had a positive TST and QFT-GIT, and both the tests were negative in six patients resulting in an agreement of 72% ($\kappa = 0.41$). Among 12 children who had been in contact with an adult having open TB, both the TST and QFT-GIT were positive in six patients and negative in two patients, respectively, resulting in an agreement of 66% ($\kappa = 0.41$) [Table 2]. Considering a positive TST of 15 mm and more in BCG-vaccinated children, both the TST and QFT-GIT were positive in 9 and negative in 12 children, respectively, resulting in an agreement of 64% ($\kappa = 0.28$).

Specificity of tuberculin skin test as compared to QuantiFERON-TB Gold In-Tube

TST specificity was only 29.6% (95% confidence interval = 14.5–50.3%) with a positive predictive value

Table 2: Agreement between tuberculin skin test and QuantiFERON-TB Gold In-Tube assay

Factors	QFT-GIT negative	QFT-GIT positive	Agreement (%)	κ
Overall				
TST negative	8	1	63	0.31
TST positive	11	13		
Children <4 years				
TST negative	2	0	37.5	0.063
TST positive	5	1		
Children >4 years				
TST negative	6	1	72	0.41
TST positive	6	12		
Contact with TB				
TST negative	2	0	66	0.41
TST positive	4	6		

QFT-GIT: QuantiFERON-TB Gold In-Tube, TST: Tuberculin skin test, TB: Tuberculosis

of 42.4% and a false-positive rate of 57.6%. Among children <4 years of age, TST specificity was only 28.6% with a positive predictive rate of 16.7% and a false-positive rate of 83.3%. Among children >4 years of age, TST specificity was 50% with a positive predictive value of 66.7% and a false-positive rate of 33.3%. In patients with contact with a patient having TB, TST specificity was 33.3% with a positive predictive value of 60% and a false positive rate of 40%. In patients with malnutrition, the specificity of TST was 42.9% with a positive predictive value of 63.6% and a false positive rate of 36.4%. In patients who had lymphadenitis, TST specificity was 45.5% with a positive predictive value of 53%.

Considering TST of 15 mm and above as positive, TST specificity increased to 63.2% and a positive predictive value was 56.3%.

DISCUSSION

Although IGRAs have been widely used for the diagnosis of latent and active TB in adults, a relative lack of validation studies in children has led to caution in their clinical interpretation. A recent meta-analysis evaluated IGRAs (QFT-G, QFT-GIT, and Enzyme Linked Immune absorbent spot [ELISPOT]) and the TST. The authors found that the sensitivities of all the three tests in active TB were similar. The pooled sensitivity was 70% for QFT-based tests, 62% for ELISPOT studies, and 71% for TST. The pooled specificity was 100% for QFT-based tests and 90% for ELISPOT but was much lower for TST (56% in all included studies and 49% in children with BCG vaccination).[30] QFT-GIT assay has been evaluated in only two studies in a pediatric population in India in which only one study has compared it with TST.[31,32] Dogra *et al.* tested 105 consecutively admitted children in whom TB was suspected or had a history of contact with an index case in a rural hospital in India and reported that the TST and QFT assay produced comparable results. BCG vaccination did not significantly affect either TST or QFT results.[31] Dayal *et al.* evaluated

the efficacy of QFT-GIT and compared the reactivity with smear, culture, and polymerase chain reaction (PCR) testing of clinical specimens. QFT-GIT showed a significantly higher sensitivity (51.2%) as compared with solid culture method for detection of TB (11.0%; $P < 0.001$), BacT/Alert three-dimensional system (12.1%; $P < 0.001$), Ziehl–Neelsen staining (19.5%; $P < 0.001$), and PCR (45.1%; $P < 0.05$). The specificity of QFT-TB-IT was 48.0%.[32] Similarly, in our study, we found that specificity was <30% in children suggestive that the false-positive rate of TST would be >50% in these children. In fact, in our patients, the chance that TST would be false positive was higher in children <4 years of age as compared to children who were older. Since children receive BCG at birth in our country, these false-positive results may have been due to BCG vaccine. A recent study in Madrid, Spain, done in immigrants or adopted children from countries that give BCG at birth found that under 3 years of age, BCG does interfere with and may cause a false-positive TST result.[33] In a meta-analysis by Farhat *et al.* which included 24 studies involving 240,203 participants who had received BCG vaccination as infants, it was found that 20,406 (8.5%) had a TST of 10 + mm attributable to BCG, but only 56/5639 (1%) were TST-positive if tested ≥10 years after BCG. In 12 studies of 12,728 participants vaccinated after their first birthday, 5314 (41.8%) had a false-positive TST of 10 + mm and 191/898 (21.2%) after 10 years. This suggests that BCG may interfere with TST till 10 years of age.[34] In those countries where the vaccine is given exclusively at birth, the BCG effect disappears after 7 years[35] and even after 4 years according to a study conducted in Canada.[36] Thus, it seems in our cohort, and TST was false positive in children <4 years of age due to BCG and maybe due to BCG even in older children. Despite this, TST remains in widespread use due to its low cost, simplicity of administration, and ease of interpretation. However, results of TST in BCG-vaccinated children may have to be interpreted with caution to prevent the overdiagnosis of TB and unnecessary treatment with ATT.

A TST result of >10 mm is taken as a positive result.[37] However, studies have suggested that induration ≥15 mm should only be considered as TB infection in the context of previous BCG.[38-41] In our study, when we considered a TST of 15 mm or more as positive, it did not make much of a change in the concordance between TST and QFT-GIT, but the specificity of TST did double. Thus, the optimal TST measurements to detect MTB in BCG-vaccinated children in endemic areas still need to be determined.

The elimination of LTBI is necessary for TB control programs. In countries where BCG vaccination is a part of the immunization schedule, IGRA would be a better choice to diagnose LTBI since TST may have false positives in vaccinated children.

Kampmann *et al.* showed a significantly lower production of IFN-γ in response to the positive control mitogen

phytohemagglutinin in children younger than 4 years old compared with children 4–15 years old ($P < 0.0001$).[42] Thus, we analyzed our data in children younger than 4 years and those 4 years and above and found that there is a difference in the concordance of QFT-GIT and TST and age in BCG-vaccinated children. Similarly, in our study, QFT-GIT results were more likely to be positive in older children >4 years of age as compared to those <4 years of age. In fact, no child had a positive QFT-GIT result <4 years of age. However, none of these children had active TB disease. These findings highlight the need for pediatric studies of larger groups of children, stratified by age.

Limitations

Our study had limitations foremost being the sample size and very few number of patients with active TB. We were thus unable to estimate the sensitivity of the QFT assay.

CONCLUSION

The concordance of TST and QFT-GIT is low in children with previous BCG vaccination and especially in children <4 years of age. QFT-GIT may help to rule out false-positive TST.

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Conflicts of interest
There are no conflicts of interest.

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