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Original article

Evaluation of QuantiFERON-TB Gold test for the diagnosis of tubercular infection in children

Anshul Dhillon [a], Reena Raveendran [b], Satish Saluja [c], Dinesh Kaul [a, *]

[a] Department of Pediatrics, Sir Ganga Ram Hospital, New Delhi, India
[b] Department of Microbiology, Sir Ganga Ram Hospital, New Delhi, India
[c] Department of Neonatology, Sir Ganga Ram Hospital, New Delhi, India

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ABSTRACT

Background: Tuberculin skin test (Mantoux test) is a known intradermal skin prick test for detection of tubercular infection in children and adult population. A new diagnostic test QuantiFERON-TB (QFT) Gold developed in 2005 and approved by the US Food and Drug Administration has good sensitivity and specificity for diagnosis of tubercular infection.

Methods: In this prospective study, 125 children who were being evaluated for tubercular infection or disease were administered Mantoux test and at the same time a blood sample was drawn for the interferon gamma estimation and evaluated by this new diagnostic assay, i.e., QFT Gold assay.

Results: Sensitivity and specificity of QFT test calculated was 96.4% and 81% respectively, whereas the negative predictive value and positive predictive value were 91.9% and 90.9% respectively. The sensitivity and specificity of the Mantoux test was found to be 89.2% and 59.5% respectively. The Cohen Kappa coefficient between the Mantoux test and QFT Gold assay for diagnosis of tubercular infection was found to be 0.627 (95% confidence interval: 0.474–0.779; $p = 0.0001$) in our study, indicating a good agreement between the two tests.

Conclusion: QFT Gold assay is an effective tool in diagnosing tuberculosis infection in a pediatric population. False positive reactions with tuberculin skin test are common as there is cross-reactivity with non-tubercular mycobacteria (NTM) and BCG vaccination. Both sensitivity and specificity of QFT test were better than those of the tuberculin skin test. Therefore, it is better than the tuberculin skin test, and the diagnostic yield is better. However, this test should not be used as gold standard for diagnosis of tubercular disease in children.

1. Introduction

Tuberculosis is a major public health concern in various developing countries. Ninety five percent of tuberculosis (TB) cases occur in developing countries where resources are unavailable for proper identification.[1] According to the World Health Organization (WHO), more than 10.3 million new cases of TB were reported in the year 2016; 6.08 million men, 3.27 million women and 1.04 million children.[2] India accounts for one-third of the total global TB burden.[3]

Latent TB infection (LTBI) occurs after inhalation of infective droplets containing the tubercular mycobacteria. A reactive tuberculin skin test (TST) and absence of clinical and radiological manifestations are the hallmark of LTBI. There is 40% increased chance of developing the disease in the subsequent years in untreated infants with LTBI.[4] It is difficult to make a diagnosis of TB in the pediatric population as children often present with vague symptoms and signs. Owing to the paucibacillary nature of the disease and difficulty in obtaining the clinical specimens, clinicians resort to an indirect approach to make a diagnosis such as history of contact, X-ray chest and TST.

Before 2005, TST was the only method for the diagnosis of LTBI. This test that uses purified protein derivative (PPD) from *Mycobacterium tuberculosis* is hampered by technical and logistical problems, false positive and false negative results, problems in administration and interpretation, difficulty in differentiation of true infection from the effect of prior BCG vaccination and infection

* Corresponding author.
E-mail address: docdineshkaul@gmail.com (D. Kaul).

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with nontubercular mycobacteria.[5] T cells that have been sensitized by prior infection are recruited to the skin where they release various cytokines that lead to induration through local vasodilatation, edema, fibrin deposition and recruitment of other cells to that area. Tuberculin sensitivity develops 3 weeks to 3 months most often 4–8 weeks after inhalation of the organisms.

Advances in genomics and immunology have led to promising alternative in vitro diagnostic tests i.e., interferon gamma (IFN-γ) assay and immunospot assay.[6–9] The former test is based on the principle that T cells of *Mycobacterium tuberculosis*–infected persons release IFN-γ when they reencounter certain TB-specific antigens[15,16] (antigens encoded within the region of difference RD1 of the mycobacterial genome not entirely specific to *M. tuberculosis complex*) and are more specific than PPD as they are not shared with BCG vaccine strains or selected non-tubercular mycobacteria (NTM) species including *M. avium.*[17,18]

It is a new in vitro test used as a diagnostic aid in detection of latent and active TB infection. With this background, we planned this study to compare the utility of IFN-γ assay (QuantiFERON®-TB Gold) with that of TST (Mantoux test) for detection of tubercular infection in ambulatory and hospitalized symptomatic children.

2. Material and methods

This was a prospective study conducted at Sir Ganga Ram hospital, New Delhi, from 1st January, 2010 to 30th June, 2011. Children aged 3 months to 18 years who attended the outpatient department (OPD) and specialty clinics and admitted in various areas of pediatrics department and who were suspected to have TB according to criteria of the TB Working Group of the Indian Academy of Pediatrics (IAP), 2004, were enrolled. Children who met the defined criteria of clinical, radiological or microbiological diagnosis of TB were eligible.

Infants younger than 3 months, hypersensitive to tuberculin protein (purified protein derivative) or Tween 80, who had received treatment for TB in the past, or whose parents/guardians refused to consent were excluded. Children infected with human immunodeficiency virus (HIV) or perinatal HIV exposure and immunecompromised children i.e., children with malignancies and/or on steroids (oral/parenteral) for more than 2 weeks were also excluded.

The enrolled children were administered TST using a 26-gauge tuberculin syringe. A volume of 0.1 ml containing 5 tuberculin units (TU) of purified protein derivative (PPD) stabilized with Tween 80 was injected intradermally. An induration of more than 10 mm after 48–72 h of administration was considered a positive TST. Three milliliters (mL) of venous blood was drawn in three one-ml heparin containing tubes. The tubes were incubated at 37 °C within 2–6 h of collection of sample. After 24 h of incubation, the tubes were centrifuged and plasma was harvested and stored at 2–8 °C. The samples were processed for IFN-γ activity using a kit designed by Cellestis Limited, Australia, and Cellestis Inc. USA (QuantiFERON®-TB Gold). The IFN-γ response was quantified by enzyme-linked immunosorbent assay. Cutoff values for a positive test were >0.35 1U/mL, as recommended by the manufacturer in the product insert. In suspected cases of pulmonary TB, sputum samples were sent for acid fast bacillus (AFB) analysis, wherever possible. In children whose sputum could not be collected, samples of gastric aspirates or bronchoalveolar lavage were used for documentation of AFB. For suspected extrapulmonary TB, samples of CSF, lymph node aspiration, ascitic fluid, and pleural fluid were processed for AFB examination. Children confirmed to have TB (bacteriological or histological evidence) or those who responded to empirical antitubercular treatment were classified as diseased subjects.[11] The study was approved by an independent institutional ethics committee. Informed consent was obtained from parents of all the enrolled subjects. The algorithm of the study has been depicted in Fig. 1.

Data were recorded in a predesigned proforma in Microsoft Excel. Statistical analysis was performed using the SPSS program for Windows, version 17.0. Continuous variables were presented as mean (standard deviation [SD]) or median (interquartile range), and categorical variables were presented as proportions or percentage. Nominal categorical data between the groups were compared using Chi-square (χ^2) test or Fisher's exact test as appropriate. Agreement between TST and QFT Gold was assessed by the percentage agreement and the Kappa (K) statistics.[10] The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), along with their 95% confidence intervals, were calculated to report the diagnostic accuracy of the two tests. A p value less than 0.05 was considered as significant.

3. Observations and results

The characteristics of enrolled subjects are given in Table 1. The mean age (SD) of the study population was 9.18 (4.8) years; 70 (56%) patients were males. Fever for more than 3 weeks duration was the commonest clinical symptom in 91 (72.8%) children. Seventy children (56%) of the enrolled subjects had a history of cough for more than 3 weeks. The most common chest radiological findings in our study were infiltrative lesions seen in 18 (60%) patients. Pleural effusion and infiltrative lesions were seen in three (10%) patients. Thirteen (10.4%) children had a history of contact with a known case of TB. These contacts had been treated for TB (less than 2 years duration) or were currently on treatment. Acid-fast bacilli on smear examination or on culture or histopathological evidence for TB was observed in 13 (10.4%) cases.

The diagnostic accuracy of TST and QFT Gold are given in Table 2. The sensitivity, specificity, PPV and NPV of TST and QFT Gold along with their 95% CI, were 89.2 (80.4–94.4), 59.5 (44.8–72.9), 81.3 (71.5–88.4) and 73.5 (55.3–86.4) and 96.4 (89.5–99.2), 81 (66.4–90.3), 90.9 (82.3–95.7), and 91.9 (76.9–97.8), respectively. The Cohen's kappa coefficient (95% CI) for agreement between QFT Gold test assay and TST for the diagnosis of TB in our study was 0.627 [(0.474–0.779), $p < 0.0001$], indicating moderate agreement between these two tests.

4. Discussion

This prospective study was carried out to evaluate the utility of whole-blood IFN-γ assay (QuantiFERON-TB Gold®) for the diagnosis of TB infection in children aged between 3 months and 18 years, as well as the comparison between the QFT Gold test and TST for the diagnosis of tubercular disease in children.

In our study, the sensitivity of TST and QFT Gold was estimated to be 89.2% and 96.4%, respectively. However, there was no statistically significant difference between them (p value, 0.072). There are few studies in which sensitivities of TST and QFT Gold test have been compared.[13–15] In a study conducted in Abuja (Nigeria), among children at high risk of latent TB (who were in contact with adult cases of pulmonary TB), disagreement between the two tests was due to negative TST and positive QFT Gold, which is indicative of higher QFT Gold sensitivity.[13] It showed that sensitivities of TST and QFT Gold are comparable. They also reported the sensitivity of TST and QFT Gold to be 90% and 92%, respectively. In another prospective study conducted in Melbourne, Connell et al.[14] found that TST was more sensitive than QFT Gold for detection of TB infection. This study's findings were in contradiction to other studies.

Specificities of TST and QFT Gold in our study were 59.5% and 81.0%, respectively. There was a statistically significant difference between them (p value, 0.032). Several studies have shown QFT Gold test to be more specific than TST.[16–21] In a prospective study by Lighter et al.,[17] 207 children receiving health care in New York

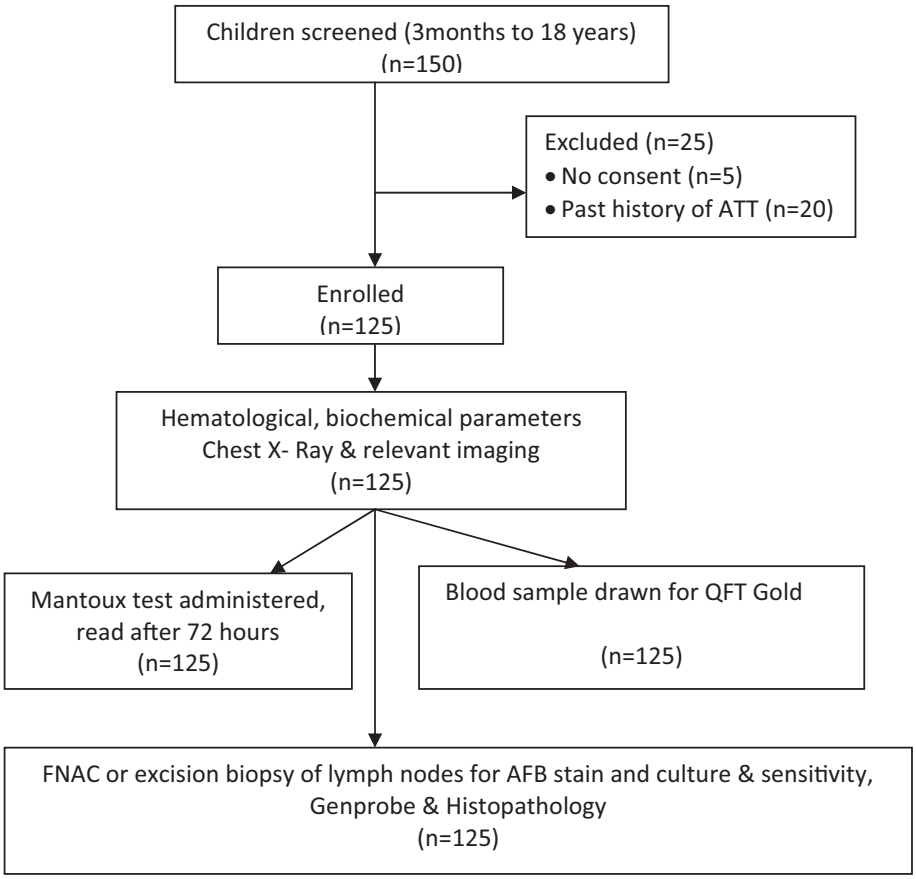

Fig. 1. Study flow chart. QFT = QuantiFERON-TB; AFB = acid fast bacillus; ATT = antitubercular therapy; and FNAC = fine needle aspiration cytology.

were assessed, QFT Gold was positive in only 23% of children with a positive TST, and positive QFT Gold was associated with *M. tuberculosis* exposure suggestive of superiority of QFT Gold specificity. However, this study pointed out that sensitivity of QFT Gold test was lower than that of TST.[17] Bianchi et al.[16] reported in a study on 336 children at risk of tuberculosis in Italy that the number of positive TST and QFT Gold results was 58 and 60, respectively. Half of these children (22/44) with a positive TST had a negative QFT Gold. These results suggest that QFT Gold is more specific and TST is more sensitive.[16]

Table 1
Baseline characteristics of study subjects (N = 125).

Characteristics	
Age, years	9.18 (4.84)
Gender: male, n (%)	70 (56)
Symptoms and signs	
History of contact	13 (10.4)
Malnutrition	53 (42.4)
Fever more than 3 weeks	91 (72.8)
Cough more than 3 weeks	70 (56)
Lymphadenopathy	17 (13.6)
BCG at birth	113 (90.4)
Chest X-ray findings	30 (24)
Cavitatory and infiltrative	18 (14.4)
Pleural effusion and infiltrative	9 (5.6)
Infiltrative	3 (2.4)
AFB culture or histopathology positive for tuberculosis	13 (10.4)
Designated	
Tuberculosis positive	83 (66.4)
Tuberculosis negative	42 (33.6)
Tuberculin positive (10 mm)	92 (72.8)
QuantiFERON-TB Gold test positive	83 (66.4)

Data expressed as number = n (%).

QFT Gold has been shown to be more closely associated with exposure to TB in children younger than 5 years than TST. Chung et al. indicated that QFT Gold is better at detecting more recent infection than TST.[17] In a study carried out by Tsiouris et al. (2006) in Gugulethu, South Africa, 184 children at risk of TB were administered both TST and QFT Gold test; overall, they found 43% children to be TST positive and 32% to be QFT Gold positive. An increasing agreement and kappa (κ) between two tests were observed with increasing TST cutoff values. This finding that kappa (κ) increased with an increased cutoff is suggestive of QFT Gold having high specificity at lower TST cutoffs.[22]

Results in our study show that QFT Gold is equally sensitive in diagnosing tubercular disease as compared to TST (p value = 0.072). However, it is more specific than TST (p value = 0.032). Although Kampmann et al.[23] and Bramford et al.[24] showed that QFT Gold did not perform significantly better than TST in identifying active tubercular disease, they found that combining these two improved the test sensitivity with both reporting an improved sensitivity of 91% compared with QFT Gold sensitivity of 89% and 78% in two studies, respectively, when QFT Gold was used in isolation. This aspect has not been analyzed in our study.

All enrolled subjects were subjected to TST and QFT tests and agreement between TST and QFT Gold showed kappa coefficient of 0.627, showing good correlation between these two tests (Table 3). Both the tests were associated with the occurrence of the disease (p value < 0.0001).

Studies have shown that the agreement of QFT Gold and TST in children range from 0.5 to 0.8.[15,25,26] A study conducted in a pediatric population in India by Dogra et al. has revealed good agreement between these two tests (kappa coefficient κ = 0.73).[15] It is

Table 2
Comparative sensitivity, specficity, PPV and NPV of tuberculin and QuantiFERON-TB Gold test.

	Sensitivity	Specificity	PPV	NPV
Tuberculin test	89.2 (80.4–94.4)	59.5 (44.8–72.9)	81.3 (71.5–88.4)	73.5 (55.3–86.4)
QuantiFERON-TB Gold test	96.4 (89.5–99.2)	81 (66.4–90.3)	90.9 (82.3–95.7)	91.9 (76.9–97.8)
p value	0.072	0.032	0.064	0.039

Data in parenthesis is 95% confidence intervals.
PPV = positive predictive value; NPV = negative predictive value.

Table 3
Correlation between the tuberculin skin test and QuantiFERON-TB Gold test in study subjects.

TST result	QuantiFERON-TB Gold test results		p value	Kappa (95% confidence interval)
	QFT Gold +	QFT Gold −		
TST >10 mm (positive)	80	11	<0.0001	0.627 (0.474–0.779)
TST <10 mm (negative)	8	26		
Total	88	37		

TST = tuberculin skin test; QFT = QuantiFERON-TB.

also concordant with our study ($\kappa = 0.62$), showing good correlation between these two tests.

Children in our study had various patterns of radiological presentation in the form of infiltrative (60%) and cavitatory (30%) lesions. However, pleural effusion plus infiltrative lesions were seen in three (10%) children. This is in contrast to a prospective study conducted by Krysl et al. on 158 patients which revealed pulmonary infiltrates in 126 patients (80%). Cavitation was present in 30 patients (19%).[12] High incidence of cavitatory lesions may be attributable to late presentation of children who had advanced to this stage of TB as seen on chest radiographs, or there could be other contributing immunological factors.

The findings in our study indicate that the QFT Gold assay is a sensitive as well as specific way to diagnose active TB infection. It was found to identify individuals with active as well as latent TB. Both sensitivity and specificity were higher than those of TST. For patients with suspected disease, the QFT Gold assay holds promise as a useful adjunctive diagnostic tool for the rapid diagnosis of TB, when the conventional diagnostic methods fail.

5. Drawbacks of the study

It is a difficult task to collect sputum samples in the pediatric population. Biological samples such as sputum, bronchoalveolar lavage, gastric aspirate and lymph node tissue/aspirate could be collected (sent for TB culture and histopathology) for confirmation of TB from only 37 enrolled subjects. AFBs were isolated in only 13 subjects. This is a small sample size of patients with confirmed diagnosis of TB. Thus, a larger sample size is required to further evaluate this assay in the pediatric population.

Conflicts of interest

Authors have no conflict of interest.

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