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Yield of QuantiFERON-TB gold in tube assay and tuberculin skin test in healthy persons from a tuberculosis endemic population

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Abstract. We assessed the yield of the QuantiFERON-TB Gold in tube test (QFT-GIT) and the tuberculin skin test (TST) in healthy persons from our general population, where tuberculosis is endemic and Bacillus Calmette-Guérin is mandatory. The yield of QFT-GIT (2%) and TST (1%) were very low among children, where the test was positive in 42% and 24% of adults, respectively. Our study results show that QFT-GIT and TST are not influenced by prior Bacillus Calmette-Guérin vaccination. Applying the reduced cut-off point of 0.13 IU/mL is adequate in a pediatric population. Moreover, both the TST and the QFT-GIT demonstrated a low yield in the pediatric population, which suggests that either TST or QFT-GIT alone or in combination can be used to diagnose *Mycobacterium tuberculosis* infection in our setting.

Keywords: Tuberculosis, diagnosis, QuantiFERON-TB Gold in tube, healthy children, endemic population

1. Introduction

Children have an accelerated progression of tuberculosis (TB) disease and a higher risk for mortality compared to adults [1]. Early and rapid detection of infection with Mycobacterium tuberculosis and TB disease is critical to enable the initiation of prophylaxis for latent TB infection (LTBI) and anti-tuberculosis therapy for children with TB disease.

However, the diagnosis of both latent and active TB is more challenging in children. Bacteriological confirmation is available only in a minority of children and radiological examination is often non-specific. Hence, the active TB diagnosis relies on the clinical presentation of the suspected child and his TB contact history [2].

The tuberculin skin test (TST) is the most widely used test for LTBI diagnosis. Although TST is not able to differentiate latent and active TB, it is used as a supplementary test for childhood TB diagnosis. However, major drawbacks to using TST are its false positivity due to the cross-reaction with Bacillus Calmette-Guérin (BCG) and other environmental mycobacteria, and false negativity in young children and immune suppressed cases [3].

Recently, *in-vitro* based immune tests have been introduced for the replacement of TST. These tests work based on the interferon-gamma (IFN-γ) secretion [so called IFN-γ release assay (IGRA)] in response to *M. tuberculosis* specific antigens such as early secretory

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antigenic target 6 kDa and culture filtrate protein 10. These tests have been validated in BCG vaccinated healthy subjects in low endemic countries and studies have demonstrated that IGRAs are less influenced by prior BCG vaccination and environmental mycobacteria than TST, and therefore have a higher specificity [4]. In most low endemic countries, IGRAs have been incorporated as the routine test for the diagnosis of TB infection.

However, the reports on the performance of IGRA from TB endemic countries are limited; hence, TST is still being used. No studies have assessed the specificity of IGRA in TB endemic countries like India [5].

In this study, we assessed the yield of the Quanti FERON-TB Gold in tube test (QFT-GIT), one of the IGRAs, and TST in healthy children. We also recruited adults in the same area to compare test performance with children. This approach will help to assess the yield of the QFT-GIT and the TST to identify individuals infected with TB.

2. Materials and methods

This study was approved by the Scientific Advisory Committee and the conduct of the study was approved and monitored by the Institutional Ethical Committee of Tuberculosis Research Centre. Written, informed consent was obtained from all the study subjects before drawing blood. If the study subject was a child, consent was obtained from parent or legal guardian.

The study participants were enrolled from offices, schools, colleges and slum areas in Chennai city, India and near by villages. The estimated incidence of TB disease in this setting was 168/100,000/year in 2007, which was classified as high TB endemic area by World Health Organization [6].

Apparently normal healthy children ($\leqslant$ 15 years) and adults ($>$ 15 years) were included. Individuals with previous history of TB or having known contact history of TB or who were malnourished were excluded from the study. All study participants were asymptomatic and had no abnormalities on chest X-ray, and were identified as healthy by a clinician during recruitment. After registering the eligible subjects, blood was drawn for total blood count, human immunodeficiency virus serology and QFT-GIT. The blood samples were taken to the laboratory within 2 h of phlebotomy and the QFT-GIT was carried out, as per the manufacturer's instructions (Cellestis Ltd, Victoria, Australia) [7]. After drawing blood samples, TST was carried out with 2 tuberculin units of purified protein derivate RT23 (Staten Serum Institute, Copenhagen, Denmark). Three sputum samples were collected from all the of study subjects for smear microscopy and *M. tuberculosis* culture, to confirm the absence of active TB infection.

The QFT-GIT test results were interpreted using the software provided by the manufacturer (Cellestis Ltd., Victoria, Australia) and the cut-off point for the diagnosis was determined as per manufacturer's instructions. If the IFN-γ secretion in response to TB antigen was $\geqslant$ 0.35 IU/mL, after subtracting the nil control IFN-γ (i.e. background IFN-γ value), it was considered positive for QFT-GIT; if the value was $<$ 0.35 IU/mL, it was considered negative. If the negative result was associated with poor phytohemagglutinin response (i.e. if IFN-γ secretion in response to phytohemagglutinin, after subtracting the nil tube IFN-γ was $<$ 0.5 IU/mL), it was considered as an indeterminate or invalid result for QFT-GIT. The difference between the two tests was calculated using the chi-square test or Fisher's exact test.

3. Results

A total of 246 persons were recruited for this study. Their age ranged from 2–85 years with a mean of 25 years. All of the study participants were seronegative for human immunodeficiency virus infection. The results of QFT-GIT and TST are given in Table 1. None of the participants yielded indeterminate results for QFT-GIT.

A total of 83 participants were children aged $\leqslant$ 15 years and 61% of them were males. The BCG scar status was indeterminate for three (4%) subjects. In the remaining 80 children, a BCG scar was present in 62 (78%) and absent in 18 (22%). Of the 83 children, two (2%) were positive for QFT-GIT and one (1%) was positive for TST at the 10 mm cut-off point. Two children, aged 14 and 15 years of age, were positive for QFT-GIT and negative for TST. One 6 year old child was positive for TST and negative for QFT-GIT. All children with only a QFT-GIT or TST positive test had been vaccinated with BCG.

The range of TB antigen specific IFN-γ secretion after subtracting the nil tube control (net TB specific IFN-γ response) in QFT-GIT negative individuals was 0–0.21 IU/mL and the mean was 0.02 IU/mL. For the 81 children with QFT-GIT negative tests, the TB specific IFN-y secretion ranged from 0–0.12 IU/mL for 79 children, and was 0.21 and 0.16 IU/mL for the 2

Table 1
Positivities of QuantiFERON-TB gold in-tube and tuberculin skin test in healthy subjects

Characteristics	QuantiFERON		Tuberculin skin test		QuantiFERON vs. tuberculin skin test P value
	n	Number of positive n (%)	n	Number of positive n (%)	
Children ($\leqslant$ 15 years)	83	2 (2)	83	1 (1)	Not significant
$\leqslant$ 9 years	46	0 (0)	46	1 (2)	Not significant
10–15 years	37	2 (5)	37	0 (0)	Not significant
Male	51	1 (2)	51	0 (0)	Not significant
Female	32	1 (3)	32	1 (3)	Not significant
Bacillus Calmette-Guérin vaccinated	62	2 (3)	62	1 (2)	Not significant
Bacillus Calmette-Guérin non- vaccinated	18	0 (0)	18	0 (0)	Not significant
Bacillus Calmette-Guérin status indeterminate	3	0 (0)	3	0 (0)	Not significant
Adults ($>$ 15 years)	163	68 (42)	134	32 (24)	$<$ 0.001
16–25 years	69	17 (25)	55	9 (16)	Not significant
26–40 years	49	17 (35)	35	10 (29)	Not significant
$>$ 40 years	45	34 (76)	44	13 (30)	0.002
Male	92	39 (42)	81	18 (22)	0.015
Female	71	29 (41)	53	14 (26)	0.014
Bacillus Calmette-Guérin vaccinated	63	30 (48)	62	13 (21)	0.029
Bacillus Calmette-Guérin non – vaccinated	39	16 (41)	35	8 (23)	Not significant
Bacillus Calmette-Guérin status indeterminate	61	22 (36)	37	11 (30)	0.01

The cut-off point of 10 mm was used for tuberculin skin test (2 tuberculin units purified protein derivative).
The difference between tuberculin skin test and QuantiFERON-TB gold (in tube) was tested in subjects for whom both results were available using chi-square t test of Fisher's exact test.

remaining children. When the cut-off point was reduced to 0.13 IU/mL as suggested by Harada et al. [8], QFT-GIT had a 5% positive yield.

A total of 163 participants were $>$ 15 years of age. QFT-GIT results were available for all adults, but TST results were only available for 135 adults, as some refused to undergo testing or failed to return for reading. QFT-GIT was positive in 42% [95% confidence interval (CI): 34%–50%], whereas TST was positive in 20% (95% CI: 13%–27%) of adults. The yield of QFT-GIT was significantly higher than TST ($P < 0.001$). The proportion of positive results using QFT-GIT or TST was significantly higher in adults than children ($P <$ 0.001). When the adult subjects were classified based on the age (16–25 years, 26–40 years and $>$ 40 years), we found that the yield of both TST and QFT-GIT increased with age. QFT-GIT was positive in 25% (95% CI: 15%–35%), 35% (95% CI: 22%–48%) and 76% (95% CI: 64%–88%) of subjects in the age range of 16–25, 26–40 and $>$ 40 years, whereas TST was positive in 16% (95% CI: 6%–26%), 29% (95% CI: 14%–44%) and 30% (95% CI: 16%–44%) of subjects respectively at 10 mm cut-off point. The QFT-GIT yield was significantly higher in subjects $>$ 40 years than TST ($P = 0.02$).

4. Discussion

We report in this study that the yield of QFT-GIT (2%) and TST (1%) was very low for healthy children, but much higher in adults at 42% and 24%, respectively. Neither QFT-GIT nor TST results were influenced by prior BCG vaccination. To our knowledge, this is the first study evaluating QFT-GIT positivity among healthy children and adults in a TB endemic country.

Assessing the annual risk of TB infection in children $<$ 9 years of age is an indirect, but easier way to estimate the TB burden in a population for which TST has been used [9]. However, it is always difficult to interpret TST results in high endemic countries, because of BCG vaccination and higher prevalence of environmental mycobacteria [3]. We also assessed the positivities of TST in our pediatric population. Among the 36 BCG vaccinated children ($<$ 9 years; negative for QFT-GIT) tested in this study, only one participant was positive for TST. Even in the children aged between 9 years and 15 years and in adults, yield of the TST did not significantly differ between BCG vaccinated and non-vaccinated subjects. An earlier tuberculin survey conducted in this population also failed to find a significant difference in the proportion of TST positive results between BCG vaccinated and non-vaccinated children [10–12]. As a result, we suggest that the effect of BCG on TST results in a population is very minimal. Unlike developed countries, BCG is given only once at birth and not repeated. This may be one of the reasons for the lessened impact of BCG on TST in developing countries [13].

When comparing the performance of QFT-GIT and TST in our study group, we found that the number of TST positive adults was significantly lower than the number of adults who had a QFT-GIT positive result. In sub-group analysis, we found the yield of QFT-GIT increased over age and highest positive yield of 76% was obtained in subjects aged $>$ 40 years. The yield of TST also increased over age but the increase was found to be lesser than QFT-GIT. In particular, TST failed to pick up 46% of QFT-GIT positive subjects aged $>$ 40 years. Logically, being infected with TB in endemic countries may be higher among the elderly compared to younger persons due to a longer exposure period. This may explain the higher yield of QFT-GIT and TST in older ages. However, the poor yield of TST in subjects $>$ 40 years suggests the an age-specific anergy for TST [2] and questions the reliability of using TST for LTBI diagnosis in elder subjects.

A previous study conducted using enzyme-linked immunosorbent spot assay in Mumbai city (another major city in India) reported that 80% of healthy adults (age range 18–70 years; mean 47 years) were positive for *M. tuberculosis* infection [14]. In contrast, our study results show only 42% of QFT-GIT positivity in adults. The difference in the IGRA methodology and the demographic characteristics of cohorts, particularly age, may explain the variation in the IGRA yield between the two studies.

Interestingly, the yield of QFT-GIT and TST were both low among children, which is similar to performance of these tests in low endemic countries. In high endemic countries, due to the high number of smear positive cases, persons may be infected not only through close contact with TB patients in the family, but also from TB patients in the community. In our setting, all children $<$ 15 years of age were negative for QFT-GIT; it can be presumed that their infection from the community is very minimal. Hence, children ($<$ 15 years) with QFT-GIT positivity increase the pretest probability for active TB.

In addition, the range of TB antigen specific IFN-γ secretion in QFT-GIT negative children was small (0–0.21 UL/mL) and most of the values were accumulated between 0 and 0.12 IU/mL. Hence, we calculated the yield of QFT-GIT when using 0.13 IU/mL as a cut-off point based on an earlier study conducted in low endemic country [8]. Even at the reduced cut-off point of 0.13 IU/mL, the positive yield QFT-GIT remained low (5%). Hence, we suggest to evaluate use 0.13 IU/mL as a cut-off point for QFT-GIT, even among children in high endemic countries. However, this cut-off point should not be used in the adult population due to the high positivity of QFT-GIT.

It is very difficult to rule out all the extrapulmonary TB cases using clinical, radiological and bacteriological examinations alone. Hence, our study cohort might have extrapulmonary TB cases. Due to this limitation, further studies are required to confirm our study results.

In conclusion, we found that QFT-GIT and TST are yield not greatly affected by prior BCG vaccination. Moreover, the low through obtained both TST and QFT-GIT in pediatric population suggests that either TST or QFT-GIT, alone or in combination can be used to diagnose the *M. tuberculosis* infection in our setting.

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