

Validation and Clinical Application of Interferon-Gamma Release Assay for Diagnosis of Latent Tuberculosis Infection in Children

Abstract

Background: India has the highest tuberculosis (TB) burden, accounting for one-fifth of the global incidence and two-third of the cases in Southeast Asia with an estimated 1.9 million new cases every year. Identifying and treating latent TB infection (LTBI) can reduce the risk of development of active disease by up to 90%, thereby decreasing a major burden to the prevalence of the disease, and thus reducing potential sources in future. **Aim:** Early diagnosis of LTBI by tuberculin skin test (TST) and a newer interferon-gamma release assay (IGRA). **Materials and Methods:** Seventy-seven clinically asymptomatic household contacts (≤ 18 years) of confirmed pulmonary TB patients were enrolled to compare the performance of TST and IGRA to diagnose LTBI. At baseline, all participants underwent testing for IGRA and TST. **Results:** TST showed positivity of 22%, while IGRA demonstrated positivity of 40% in the diagnosis of latent TB. Kappa value at 95% confidence interval was 0.4753, indicates a moderate agreement between the two tests. This indicates that IGRA is a better predictor of latent TB. Maximum positive percentage was in the age group of 16–18 years in both the tests followed by 1–5 years. **Conclusion:** IGRA is a better predictor or diagnostic marker of LTBI. Although TST remains the preferred method of testing LTBI, IGRAs like ETB-Feron; a more efficient diagnostic test can be preferred based on patient affordability, logistics, and individual preferences.

Keywords: *Interferon-gamma release assay, latent tuberculosis infection, tuberculin skin test, tuberculosis*

Introduction

Tuberculosis (TB) is one of the major causes of morbidity and mortality in tropical countries. The World Health Organization estimates, approximately one-third of the population in the world is affected by TB.^[1] India has the highest TB burden, accounting for one-fifth of the global incidence and two-thirds of the cases in Southeast Asia with an estimated 1.9 million new cases every year.^[2] India, the world's second most populous country, accounts for a quarter of the world's annual incidence of TB. Every year around 2 million people develop TB in India and 300,000 die of it.^[3] Children account for 5%–15% of total TB cases worldwide. In majority of the population, infection with *Mycobacterium tuberculosis* is contained by the host defenses and infection may either remain latent or get cleared. However, latent infection can become active at any time, hence identifying and treating these

latent TB infections (LTBIs) can reduce the risk of development of active disease by up to 90%, thereby decreasing the major burden to the prevalence of the disease and thereby reducing potential sources in future.^[4]

The contacts of TB patients are the vulnerable population who are at risk of developing TB. A recent study in India showed that 8.7% of household contacts were diagnosed with TB.^[5] Particular attention should be paid to contacts with the highest susceptibility to TB infection and subsequent development of active disease, especially in the pediatric age group and the immunosuppressed people.^[6] As the success of global TB elimination depends on achieving national TB control programme goals in high TB burden countries, it opens up a debate on whether LTBI should be treated along with active TB cases.^[7] LTBI is defined as “a state of persistent immune response to stimulation by *M. tuberculosis* antigens with no evidence of clinically manifest active TB.” The global burden

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Received: 11 March, 2019.
Revision: 12 April, 2019.
Accepted: 16 August, 2019.
Published: 11 October, 2019.

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Access this article online

Website:
www.ijabmr.org

DOI:
10.4103/ijabmr.IJABMR_86_19

Quick Response Code:



How to cite this article: Benachinmardi KK, Sangeetha S, Rao M, Prema R. Validation and clinical application of interferon-gamma release assay for diagnosis of latent tuberculosis infection in children. *Int J App Basic Med Res* 2019;9:241-5.

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of LTBI is not known, as there is no definitive test for its diagnosis. The current modalities for diagnosing LTBI are tuberculin skin test (TST) and since the discovery of the ESAT6 antigen, Interferon-Gamma Release Assays (IGRAs).^[8] As there is no gold standard test for diagnosing LTBI, sensitivity and specificity of these tests cannot be determined. But diagnosing LTBI in children, as compared to adults can have more beneficiary effect or results, as children are most likely to have been infected recently as compared to adults who might have been infected decades earlier. This will increase the predictability rate of TST and IGRA as well. Household contacts are at higher risk of developing LTBI when compared to the general population.^[9] There are comparatively few studies reported in literature on diagnosis of LTBI in children and hence this study was conducted to investigate the predictive value of TST and IGRA in diagnosis of LTBI in pediatric household contacts of confirmed pulmonary TB cases.

Materials and Methods

This cross-sectional study was carried out in the Department of Microbiology at RRMCH, Bengaluru, India which is a large tertiary care center located in Bengaluru, South India, with a containment area of several adjacent regions. The study was approved by the Institute Ethics Review Board. Written informed surrogate consent was obtained from parents or legal guardians of all the participants of this study.

In this study, newly diagnosed sputum-positive pulmonary TB cases were first identified from the Outpatient Department of TB and Chest Disease department and Directly Observed Treatment Short-course (DOTS) center of our institute and various other surrounding DOTS centers of Bengaluru region. Simultaneously, their close clinically asymptomatic household contacts were enrolled in the study from January 2018 to 2019. For the present study, clinically asymptomatic household contacts of pulmonary TB patients were defined as an extended group of family members who were ≤ 18 years, residing together with the pulmonary TB index case in the same household for more than 3 months and having a common sleeping area at night.

In an index case, pulmonary TB was confirmed by sputum smear microscopy and Cartridge-Based Nucleic Acid Amplification Test as per the National TB Guidelines.^[10] Household contacts of these patients who were < 18 years without any signs and symptoms of active TB were included in the study.

On their first visit, blood samples were collected for STANDARD ETB-Feron enzyme-linked immunosorbent assay (ELISA) (E B-Feron from SD Biosensor, Republic of Korea), and subsequently, the tuberculin was administered intradermally into the middle one-third of volar surface of forearm. The TST was done using standardized 2 tuberculin units of purified protein derivative (PPD), RT23 (from

Arkray Healthcare Pvt Ltd, Surat, India) and the results were interpreted after 48 h up to 72 h by measuring the size of the induration (mm). The cutoff for TST was 5 mm induration.^[11]

The STANDARD E TB-Feron assay was performed as per the manufacturer's instructions. For this assay, three specialized blood collection tubes, namely: the nil control, TB antigen tube, and mitogen tube available with the test kit were utilized. Peripheral venous whole blood (3 ml) was collected through venipuncture from each participant directly into these three tubes of 1 ml capacity each. These tubes were gently shaken to ensure thorough mixing of the blood with the content of the tubes. The TB antigen tube contained *M. tuberculosis*-specific antigens ESAT-6, CFP-10, and TB 7.7 for stimulating the cytokines in blood samples. The mitogen contained phytohemagglutinin served as positive control. Tubes were incubated at 37°C (within 6 h of venipuncture) for 16–24 h. Following incubation, plasma was harvested from the blood samples through centrifugation (2000–3000 relative centrifugal force) for 15 min. The gel plugs present inside the tubes facilitated separation of the plasma from the blood cells. The plasma was collected and stored in properly labeled Cryo Vials at -70°C till future use. The level of interferon- γ (IFN- γ) in plasma samples was estimated through ELISA kits (with recombinant human IFN- γ standard) provided with assay packaging. The kit manufacturer's protocol was followed. Proper thawing of the plasma samples was carried out, and ELISA was performed in an automated processor in batches. Test results were interpreted as positive, negative, or indeterminate as per kit manufacturer's instructions.

Statistical analysis

Data are presented as means \pm standard deviations for continuous variables and as frequencies (with percentages) for categorical variables. Continuous variables were compared using independent samples *t*-tests, and categorical variables were compared using Pearson's Chi-square test or Fisher's exact test. The Kappa statistic (*k*) was used to evaluate the concordance between TST and IGRA.

Results

A total of 77 clinically asymptomatic household contacts of 36 microscopically confirmed sputum-positive TB index patients were enrolled in the study. The contacts belonged to age group of 2.5–18 years. Age distribution of the contacts is as shown in Figure 1. Male-to-female ratio was 0.63. Maximum numbers of contacts were in the age group of 6–10 years with more occurrences at the mean age of 9.6 years.

Baseline characteristics of household contacts of patients with pulmonary TB are as shown in Table 1. TST was positive in 22% and ETB-Feron was positive in 40% of the contacts. Positive percentage was highest in the age group of 16–18 years followed by 1–5 years. However, difference

in positive percentage between TST and ETB-Feron was highest in the age group of 1–5 years followed by 11–15 years. Positive percentage is as shown in Table 2.

There was moderate agreement between the two tests, i.e., TST and IGRA with a Kappa value of 0.4753 (95% confidence interval) making the IGRA (E TB-FERON) test statistically significant. Agreement between the two tests is as shown in Table 3.

Discussion

It is a well-known fact that, infection with *M. tuberculosis* does not eventually lead to development of active TB. The

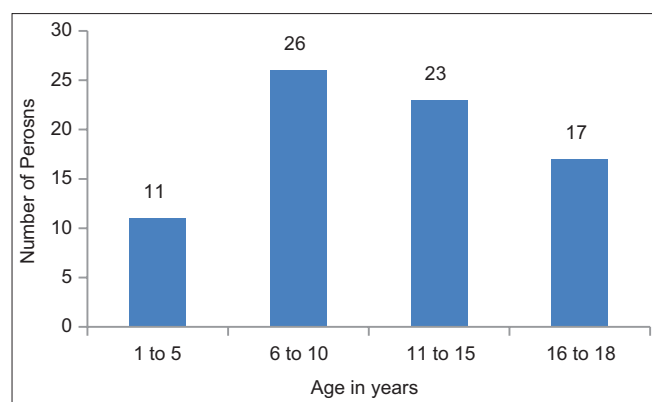


Figure 1: Age distribution of contacts

Table 1: Baseline characteristics of household contacts (n=77)

Variables	Household contacts, n (%)
Age (years)±range	10.74±4.50
Gender	
Male	30
Female	47
TST	
Positive	17 (22)
Negative	60 (78)
TB-Feron	
Positive	31 (40)
Negative	46 (60)
Indeterminate	00

TST: Tuberculin skin test; IGRA: Interferon Gamma Release Assay; TB: Tuberculosis

risk for an individual with LTBI in his lifetime to progress to active TB is estimated to be 5%–10%.^[12] This risk is especially high among pediatric population and possible immune-compromised states.^[13]

The preventive treatment for this population is risky and costly as well. Moreover, the treatment should be aimed selectively at the high-risk population who will progress to active TB disease. Only they would benefit from treatment for LTBI. A critical component toward this end is timely diagnosis.^[14]

There is no gold standard to diagnose LTBI. TST and IGRA provide reasonable options, but they both require a healthy immune response from the individuals. Although both these tests are not perfect in measuring progression to active disease, reviews show that IGRAs do well in this direction as compared to TST.^[14]

Although TST was developed by Charles Mantoux in the year 1907, it has its significance as the best prognostic marker of active TB and the best diagnostic marker of LTBI since 1890. In cases of extrapulmonary TB, TST is one of the recommended and trusted tests along with others. Although TST is the oldest and cheapest test available to diagnose LTBI, it has many disadvantages such as cross-reaction of antigens used in PPD with Bacillus Calmette–Guerin (BCG) and several nontuberculous mycobacteria (NTM). Hence, it has lower specificity in high TB burden settings like India where there is high BCG coverage and also its sensitivity is compromised in immunosuppressed individuals, in advanced TB cases and the malnourished.^[15]

In contrast, antigens used in IGRA are TB-specific antigens which do not cross-react with any BCG substrains or NTM species. Only one blood sample is necessary and a second visit is avoided as in TST. However, IGRA is expensive; requires blood samples and sophisticated laboratories.^[16]

In our study, TST positive percentage was 22% and standard ETB-Feron positivity was 40%. There are studies which have compared agreement between TST and IGRA, rather than sensitivity and specificity, because there is no gold standard test for diagnosis of LTBI. The Kappa values, which are used to assess the agreement between

Table 2: Positive percentages of tuberculin skin test and ETB-Feron

Age group	Number of persons	TST		Positive percentage TST	ETB-Feron		Positive percentage ETB-Feron	Difference in result of positive percentage between TST and ETB-Feron
		Positive	Negative		Positive	Negative		
1-5	11	2	9	18	5	6	45	27
6-10	26	5	21	19	8	18	31	12
11-15	23	5	18	22	10	13	43	22
16-18	17	5	12	29	8	9	47	18
Total	77	17	60		31	46		

TST: Tuberculin skin test

Table 3: Agreement between tuberculin skin test and ETB-Feron assay

Result	TST		Total	Agreement κ , 95% CI
	Positive	Negative		
ETB-Feron				
Positive	15	16	31	0.5543
Negative	2	44	46	(0.687-0.263)
Total	17	60	77	

CI: Confidence interval; TST: Tuberculin skin test

the two tests, are highly inconsistent ranging from -0.03 to 0.87 . These studies had study participants who were active TB cases or HIV cases with symptoms, treated TB cases, immigrant individuals from high TB burden settings, and most importantly, all were adults >18 years.^[17-19] Our study is unique in that, we have taken pediatric age group who are more vulnerable for the development of active TB disease and are close contacts of TB cases. Our Kappa value is 0.4753 ; this value shows moderate agreement between the two tests. This indicates that IGRA is better than TST in identification or diagnosis of LTBI. These results need to be further supported by clinical follow-up of these participants over the next 2 years for development of active TB.

In a study by Sharma *et al.*,^[7] where comparison was done between TST and IGRA in diagnosis of LTBI from India, they found that IGRA Gold in-tube QuantiFERON-TB-Gold in-tube assay was marginally superior to TST with IGRA positivity of 60% as compared to 52% by TST. Another study by Mohamed *et al.*^[16] also had similar findings. Furthermore, they compared TST results at different measurements, i.e., at 5 mm, 10 mm, and 15 mm. However, as per Centers for Disease Control and Prevention guidelines, 5 mm is the cutoff for children who are recent contacts of TB patients, which was followed in our study.^[11] In the present study, TB-Feron positivity was 40% compared to 22% by TST which is statistically significant. The possible explanation for this is, only children <18 years were included in the study; they would have diminished TST response owing to altered or immature cellular immunity in this age group. It is well known that regular BCG vaccination and mucosal exposure to several nonpathogenic environmental Mycobacteria suppresses TST response and increases *Mycobacterium*-specific IFN- γ response in peripheral blood.^[20] There are studies which support findings similar to our study, that IGRAs are better predictors of LTBI when compared to TST.^[21-23] In a meta-analysis by Sun *et al.*, who compared seven studies that assessed IGRA specificity and showed pooled specificity for IGRA was 100% as compared to 56% by TST.^[24] IGRA performs differently in high TB settings when compared to low burden settings; this explains the differences seen in various studies.^[16]

A key finding in our study is IGRA+TST- discordance among children who were close household contacts of sputum-positive pulmonary TB patients. IGRA+TST-

discordance has also been reported in many other studies where recent household TB contacts, elderly, recent immigrants from TB endemic areas, immunocompromised population, and in pregnancy.^[25-29] The proposed theory for this discordance is that, TST is a delayed type of hypersensitivity reaction by Th1 immune response, wherein IFN- γ , tumor necrosis factor- α , interleukin (IL)-2, and other Th1 cytokines are also involved in causing skin induration. However, IGRA measures only IFN- γ produced in the blood in response to stimulus by TB-specific antigens. Lower IFN-gamma and IL-2 may prevent immune system from triggering a delayed type of hypersensitivity reaction, resulting in false-negative TST.^[29]

In the present study, there is an increase in the positivity percentage of TST with increasing age; however, this is not true with IGRA. However, IGRA has shown maximum positivity percentage in the age group of $16-18$ years (47%) followed by $1-5$ years (45%), $11-15$ (43%) years, and $6-10$ years (31%). In a study by Eom *et al.*,^[9] TST was positive in 42.6% and IGRA 45.7% and also there was an increase in the positive percentage with age in both TST as well as IGRA. This study had included elderly patients. Many other studies also support the findings of our study that IGRAs are better predictors of LTBI than TST in close household contacts of sputum-positive pulmonary TB patients.^[9]

Conclusion

IGRA (ETB-Feron) is a better predictor of latent TB infection. Follow-up of these contacts is required to know the development of active TB disease in them in near future in order to corroborate this.

Acknowledgment

We would like to thank Mrs Chandrakala C for statistical analysis of data and Mr. Ayub for the technical help.

Financial support and sponsorship

The kit was supplied free of cost for evaluation by SD Biosensor, Korea.

Conflicts of interest

There are no conflicts of interest.

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