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IGRA Assay Diagnostic Performance in Extra Pulmonary TB and Comparison with Serum ADA and TST

Sodani Sadhna*, Hawaldar Ranjana**

*Assistant Professor, Microbiology Deptt., MGM Medical college, Indore. **Microbiology Deptt., Sampurna Sodani Diagnostic Clinic, Indore (M.P).

Abstract

Background : TB (Tuberculosis)is the second most common cause of death from infectious diseases worldwide. Every year TB claims the lives of about 3 million people and newly infects 8million people more throughout the world. India alone accounts for 2 million active TB cases and 0.5 million deaths per year. The enormous pool of latently infected individuals poses a major hurdle for global TB control. Each person carrying latent TB infection (LTBI) has approximately a 10% chance of progressing to active disease. The rate of progression is elevated among certain groups including those who have been recently infected and those with a weakened immune response. Making the diagnosis of LTBI is constrained by low bacterial load that makes it impossible to directly detect Mycobacterium tuberculosis and a weak humoral response that makes serological testing unreliable. With the banning of TB IgG and IgM Test methodologies by WHO a few years back due to false results there was a need for a serological test for diagnosing extrapulmonary TB where no other site specific sample is available for PCR or culture. Recently interferon gamma release assays(IGRA) have shown their superior diagnostic performance over TST and ADA by using atleast two superior antigens ,Early secretory antigentarget 6(ESAT-6)and culture filtrate protein-10(CFP-10)present exclusively in Mycobacterium tuberculosis but absent in BCG strains and most non tuberculous mycobacteria[5]. It is a whole blood test that can aid in diagnosing Mycobacterium tuberculosis infection including both LTBI and active disease. *Aim*: The present study was a prospective study conducted between August 2014 to January 2015 in 110 patients coming to Sampurna Sodani Diagnostic Clinic Microbiology department, a stand alone diagnostic centre of Central Madhya Pradesh with suspicion of Mycobacterium tuberculosis infection. The study aims to analyse the performance of IGRA(Immunocheck Platinum assay , Immunoshop) in extra pulmonary TB cases and comparing it with serum ADA levels and TST. *Materials and method*: From August 2014 to January 2015, 110 patients suspected of having EPTB were included in the study. Interferon gamma release assay was performed according to manufacturer's instructions ((Immunocheck TB, Immunoshop). A positive test was defined as T-N Value of e" 14.0 but > 25% of N value, A negative result was defined as T-N value of < 14.0 or e" 14.0 but <25 % of N. Indeterminate results were excluded from the study.Simultaneously serum ADA was performed on all the 110 patients and ADA below 30 u / L was considered negative. Only 12 patients were advised TST by clinicians.For diagnostic puposes a TST with induration > 10 mm was considered positive. *Statical analysis used*: Cohen's Kappa statistical analysis. *Results:* Out of 110 patients ,31 patients were positive with both IGRA(Immunocheck TB)and serum ADA. 10 patients were positive with IGRA(Immunocheck TB) but negative with serum ADA. None of the patients showed negative results withIGRA(Immunocheck TB) but positivity with serum ADA. 69 patients were negative with bothIGRA(Immunocheck TB) and serum ADA. The correlation between the two tests was 81.26%. with K value of 0.80. ***Conclusion:*** IGRA is a new assay based on in vitro detection of IFN-Y that may replace TST as a diagnostic tool for latent and active TB especially EPTB. The tests is more specific and does not require patients to return for a second visit. Cost , technical limitations and limited data in the most vulnerable groups such as children and immuno suppressed patients may present the immediate replacement of TST.

Keywords: IGRA; Immunocheck TB;TST; Serum ADA; INF Y.

Corresponding Author: Ranjana Hawaldar, Microbiology Deptt., Sampurna Sodani Diagnostic Clinic, LG-1, Morya Centre, 16/1, Race Course Road, Opp Basket Ball Complex, Palasia, Indore, Madhya Pradesh- 452001

E-mail: drranjana@sampurnadiagnostics.com

Introduction

TB (Tuberculosis)is the second most common cause of death from infectious diseases worldwide. Every year TB claims the lives of about 3 million people and newly infects 8million people more throughout the world. India alone accounts for 2 million active TB cases and 0.5 million deaths per year.

TB is a communicable disease caused by Mycobacterium tuberculosis, which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory tuberculous disease.

The enormous pool of latently infected individuals poses a major hurdle for global TB control. Each person carrying latent TB infection (LTBI) has approximately a 10% chance of progressing to active disease. The rate of progression is elevated among certain groups including those who have been recently infected and those with a weakened immune response. Making the diagnosis of LTBI is constrained by low bacterial load that makes it impossible to directly detect Mycobacterium tuberculosis and a weak humoral response that makes serological testing unreliable.

As a consequence, for much of the previous century diagnosis of LTBI has been defined as a positive TST(tuberculin skin test) in an asymptomatic person exposed to TB with no other evidence of active disease.

The TST has been used for more than hundred years for the diagnosis of both active and latent TB [1]. The test is based on the intradermal injection of purified protein derivative(PPD), a crude mixture of mycobacterial antigens, including some from the vaccine strain Mycobacterium bovis, Bacillus Calmette Guerin (BCG) and from non tuberculous mycobacteria[2]. TST is an inexpensive test and does not require additional infrastructure to be performed, hence its popularity. However, TST has many drawbacks including a high rate of false positive results, and a high rate of false negative results among immuno compromised patients ,a need for patient compliance to return for the test to be read, and subjective and interpersonal variability among health care workers when interpreting the result[3]. TST also has the potential disadvantage of boosting an anamenestic response with successive tests[4].

Adenosine deaminase (ADA) is an enzyme widely distributed in mammalian tissues, particularly in T lymphocytes. Increased levels of ADA are found in various forms of TB making it a marker for the same. However , ADA is also raised in many other infectious diseases like infectious mononucleosis, Typhoid, Viral Hepatitis , initial stages of HIV and in many malignant tumors. Fewer studies have been conducted on the diagnostic utility of serum ADA levels in diagnosing EPTB.

With the banning of TB IgG and IgM Test methodologies by WHO a few years back due to false results there was a need for a serological test for diagnosing extrapulmonary TB where no other site specific sample is available for PCR or culture.

Recently interferon gamma release assays(IGRA) have shown their superior diagnostic performance over TST and ADA by using atleast two superior antigens, Early secretory antigentarget 6 (ESAT-6)and culture filtrate protein-10 (CFP-10) present exclusively in Mycobacterium tuberculosis but absent in BCG strains and most non tuberculous mycobacteria [5]. It is a whole blood test that can aid in diagnosing Mycobacterium tuberculosis infection including both LTBI and active disease.

IGRA results are based on the proportion of Interferon gamma (IFN-Y) released in response to tuberculin as compared with mitogen. Interpretation of IGRA results is stratified by estimated risk of infection with Mycobacterium tuberculosis in a manner similar to that used in for interpreting TST with different cut off values. A positive results suggest that MTB infection is likely, negative result suggests that MTB infection is unlikely and an indeterminate result suggests that the results cannot be interpreted as a result of low mitogen response or high background response. Each result is considered in conjuction with other epidemiological, historical, physical and diagnostic findings.

IRGA based assays can be used in all circumstances in which TST is currently being used including contact investigations, evaluation of recent immigrants who have had BCG vaccination and TB screening of healthcare workers and others who are at an increased risk of LTBI.

Advantages of IGRAs

- Requires single patient visit to draw a blood sample.
- Results are available within 24 hours.
- Does not boost responses measured by subsequent tests which can happen with TST.
- Not subject to reader bias.
- Not affected by prior BCG vaccination.

Disadvantages and limitations

- Blood samples must be processed within 12 hours of collection while WBCs are still viable.
- There are limited data on the the use of IGRAs in children younger than 17 years age, among persons recently exposed to Mycobacterium tuberculosis and in immuno compromised individuals.
- Limited data on the use of IGRAs to determine who is at increased risk of developing TB disease.

The present study was a prospective study conducted between August 2014 to January 2015 in 110 patients coming to Sampurna Sodani Diagnostic Clinic Microbiology department ,a stand alone diagnostic centre of Central Madhya Pradesh with suspicion of Mycobacterium tuberculosis infection. The study aims to analyse the performance of IGRA by Immunocheck TB assay (Immunoshop) in extra pulmonary TB cases and comparing it with serum ADA levels and TST.

Materials and Methods

From August 2014 to January 2015, 110 patients suspected of having EPTB were included in the study. Active Pulmonary TB was excluded on the basis of symptoms review and chest radiographs.

The patients included in the study were divided into 0-12 years, 13-21 years, 22 to 49 years, 50-79 years and more than 80 years of age (Table I). Out of 110 patients 54 patients were male and 56 were female patients. 41 patients tested positive and 69 were negative by IGRA(Immunocheck TB)kit. 53 patients were referred by physicians, 14 by ophthalmologists is, 9 by gynaecologists , 4 by ENT specialists, 20 by orthopaedicians, 7 by paediatricians and 3 by surgeons. The chief general complaints were low grade fever, malaise, weight loss, backache. Demographic and clinical characteristics of the study population are shown in table 2.

Interferon gamma release assay was performed according to manufacturer's instructions ((Immunocheck TB, Immunoshop). A positive test was defined as T-N Value of 14.0 but > 25% of N value, A negative result was defined as T-N value of < 14.0 or 14.0 but <25 % of N. Indeterminate results were excluded from the study.

Simultaneously serum ADA (Tulip)was performed on all the 110 patients and ADA below 30 u/L was considered negative. 30-40 suspect, >40 – 60 strong suspect and > 60 was considered positive.

Only 12 patients were advised TST by clinicians.For diagnostic purposes a TST with induration > 10 mm was considered positive.

Results

Out of 110 patients 41 patients were positive and 69 negative by IGRA.There was no significant male to female ratio in the two groups.Majority of the patients were between 22to49 years age group followed by 13 to21 years , 0-12 years and least in the age group above 80 years.

A.Comparison ofIGRA(Immunocheck TB)with serum ADA levels

Table 3 shows the comparison chart of IGRA (Immunocheck TB) with serum ADA and figure 1 shows the distribution of ADA and IGRA values.

Out of 110 patients, 31 patients were positive with both IGRA (Immunocheck TB) and serum ADA. 10 patients were positive with IGRA (Immunocheck TB) but negative with serum ADA. None of the patients showed negative results with IGRA(Immunocheck TB) but positivity with serum ADA. 69 patients were negative with both IGRA (Immunocheck TB) and serum ADA.

Stastical analysis used

Cohen's Kappa statistical analysis was performed to know the correlation between the two tests .The analysis showed an excellent correlation of 81.26%. with K value of 0.80 between the two tests.

B.Comparison of Immunocheck TB with TST

Table 4 shows comparison between IGRA (Immunocheck TB) with TST.

Out of 12 patients tested with TST as compared to 100 patients of IGRA test. 9 patients were positive with both IGRA (Immunocheck TB) and TST. None of the patients showed negative TST with a positive IGRA test. Two patients showed a positive TST while IGRA was negative. 2 patients had both IGRA and TST tests negative. The correlation between the two tests was 58%. K value was not calculated because of the limited data available for TST as only 12 patients had their TST done.

Table 1: Showing demographic distribution of patients

Total Patients : 110

AGES	MALE	FEMALE	TOTAL
0-12	09	05	14
13-21	07	09	16
22-49	32	34	66
50-79	04	08	12
>80	02	00	02

Table 2: Showing distribution of patients according to speciality and clinical signs and symptoms

SPECIALITY	NO.OF PATIENTS	CHIEF SIGNS AND SYMPTOMS
MEDICINE	53	Loss of weight,loss of appetite,low grade fever
OPHTHALMOLOGY	12	Diminution of vision,uveitis
GYNAECOLOGY	09	Infertility,recurrent abortions
ENT	04	Cervical lymphadenopathy
ORTHOPAEDICS	20	Chronic backache
PAEDIATRICS	07	Lymphadenopathy,failure to thrive
SURGERY	03	Mesenteric lymphadenopathy,pain in abdomen
TOTAL	110	

Table 3: Comparison of IGRA(Immunocheck TB) with Serum ADA levels

		ADA	
		POSITIVE	NEGATIVE
TB IGRA	POSITIVE	31	10
	NEGATIVE	0	69

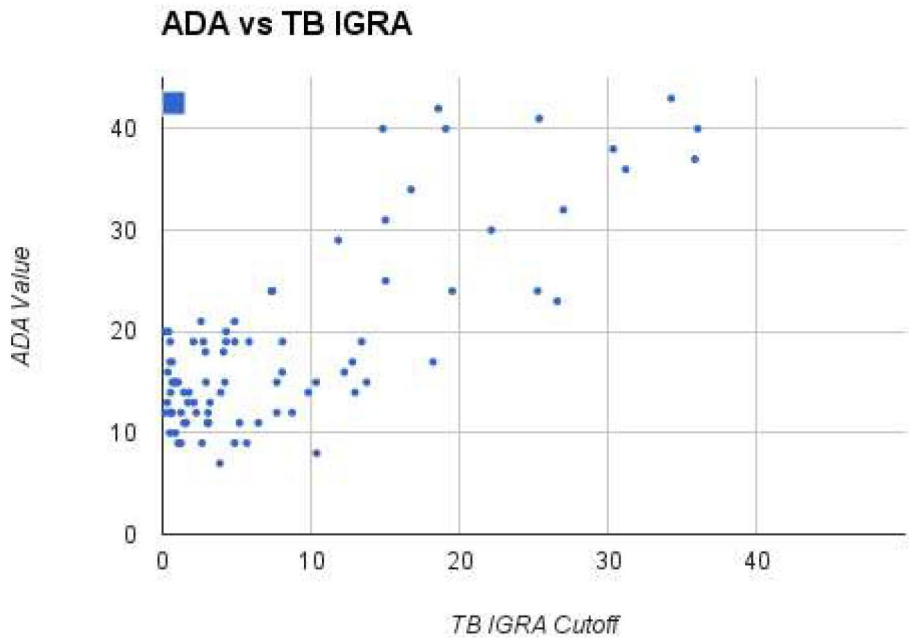
K – 0.80
Correlation results - 81.26%

Table 4: Showing comparison of IGRA(Immunocheck TB) with TST

		TST	
		POSITIVE	NEGATIVE
TB IGRA	POSITIVE	9	0
	NEGATIVE	2	2

Correlation results – 58%

Fig. 1 : showing distribution of ADA and IGRA values



Interpretation

Out of 110 patients included in the study, IGRA by Immunocheck TB(Immunoshop) and serum ADA (Tulip)was performed on all patients while only 12 patients had their TST done. Cohen's Kappa Statistical analysis was performed to assess the correlation between IGRA (Immunocheck TB) and serum ADA which showed a good correlation of 81.26% with K value of (0.80).

Correlation between IGRA and TST was only 58% probably due to very few TST data.

Fuchu Qian et al found a sensitivity of 80.9% in EPTB [16].

Miguel G. Madariaga et al studied the clinical utility of Interferon Gamma release assay in the diagnosis of TB and found an agreement between TST and ELISPOT of (K=0.72) and concluded that ELISPOT was more accurate that TST for the detection of latent tuberculosis and presumably more sensitive.[15]

Discussion

Early diagnosis of TB infection is a crucial process for the prevention and treatment of TB. At present sputum culture with microbiological confirmation is the gold standard for clinical diagnosis of active pulmonary TB, but this method takes a long time that cannot meet the clinical demand.

The diagnosis of Extra pulmonary TB becomes all the most difficult because specialy no site specific sample is available for culture and microbiological confirmation. In such circumstances IGRA methods play a crucial role in arriving at a diagnosis as compared to TST which has its own drawbacks and limitations. ADA levels have been used to diagnose TB in effusions and the present study aimed to evaluate the correlation between serum ADA and IGRA for diagnosis of EPTB which showed an excellent correlation of 81.26% with a K value of 0.80.

Conclusion

Current evidence suggests that all the three tests – serum ADA, TST and IGRA assays have advantages and limitations. All tests appear to be useful. No single test will suit all conditions. The development of IFN-Y assays have increased the diagnostics tools available for LTBI. Its important to consider all the tests as part of an expanding armamentarium of TB diagnostics. The tests to be selected will depend upon the population, the goal of testing and the resources available.

The utility of IFN-Y assays in high burden countries such as India needs further evaluation. Long term studies are needed to determine the association between Interferon gamma results and the subsequent risk of active TB.

In summary, the ELISA IGRA is a new assay based on in vitro detection of IFN-Y that may replace TST as a diagnostic tool for latent and active TB especially EPTB. The tests is more specific and does not require patients to return for a second visit. Cost , technical limitations and limited data in the most vulnerable groups such as children and immuno suppressed patients may present the immediate replacement of TST.

Although substantial progress has been made in documenting the utility of IGRAs ,further studies and research are needed.Future studies focusing on determining the value and limitations of IGRAs in situations of medical importance in TB care are needed.

According to recommendations and reports by CDC, questions which need to be addressed are

- Are IGRAs better at predicting subsequent active TB than TST?
- Are higher INFy responses associated with a greater risk for developing active TB?
- Do IGRAs perform differently in children than in adults,in both pulmonary and extrapulmonary TB?
- What magnitude of change in INFy response indicate new infection?
- What effect does treatment of M.tuberculosis infection have on IGRA results?

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