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Role of Interferon-Gamma Release Assay in Evaluation of Suspected Active Tuberculosis

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Abstract

Background: *The Global burden of TB remains enormous, despite standardized care and availability of treatment through DOTS and other standard regimens for individual treatment. To this day Tuberculosis constitutes a major public health burden affecting one-third of the world's population. We aimed the role of IGRA with QuantiFERON TB-Gold IN TUBE ASSAY, in the evaluation of suspected active tuberculosis.* ***Methods****: A total number of 53 subjects were included in the study, Complete blood picture, ESR, complete urine analysis, RBS, HIV, Serum creatinine, Blood urea, LFT were done. Wherever samples could be obtained, by suitable invasive and non-invasive methods, were subjected to histopathological examination, Bacterial and fungal cultures, and fluid analysis. All the subjects underwent a Tuberculin skin test and QuantiFERON TB Gold in Tube assay.* ***Results****: Patient with malignancy had both QuantiFERON-TB Gold In-Tube test (QFT-GIT) and TST positivity and was not started on treatment with ATT. Chest x-ray was abnormal in 24 patients (45.6%) and 54.7% of patients have a normal x-ray. The most common abnormal finding was pleural effusion in 50% and consolidation in 45.8% and lymph node enlargement of the hilar region seen in one patient. The overall Sensitivity, Specificity, PPV and NPV for QFT-GIT was 85.29%, 88.24%, 93.55% and 75% respectively similarly the TST 91.43%, 50%, 78.05% and 75%.* ***Conclusion****: A positive QFT-GIT result improved clinical evaluation of tuberculosis suspects, especially in patients where a diagnostic confirmation was not possible, viz., extrapulmonary TB, and FUO. IGRAs are superior to the TST because they are unlikely to be falsely positive. Further studies setting different cut-off values for active TB and LTBI and lower cut-off values for IGRAs in high burden countries may show realistic values for this assay in the diagnosis of active TB.*

Keywords*: Interferon Gamma Release, QuantiFERON-TB Gold In-Tube test (QFT-GIT), Tuberculin skin tests (TST)*

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Introduction

Every year there are 9 million new active TB cases and nearly 1.4 million TB deaths worldwide mostly in the poorest communities of the developing world. One-third of the world's population has latent TB which may later develop into an active form of the disease, tuberculosis also has become the leading cause of death among people with HIV. [1] The five countries that rank first to fifth in terms of the number of incident cases in 2009 are India (2 million), China (1.3 million), South Africa (0.49 million), Nigeria (0.46 million), and Indonesia (0.43 million). India and China alone account for an estimated 35% of TB cases worldwide. [1] The incidence, prevalence and mortality of TB in India was 2.3 million (185 persons/100,000), 3.1 million (256 persons/100,000) and 0.32

million (26 persons/ 100,000) respectively. HIV among estimated incident TB patients was 0.11 million, MDR-TB among notified pulmonary TB patients was 0.064 million, notified new pulmonary TB patients was 0.021 million, Notified re-treatment, pulmonary TB patients, 0.043 million. [2] Multidrug resistance also is a growing problem. A key challenge for the public health community is to be able to effectively diagnose patients so that valuable resources and medicines are not wasted on misdiagnosis and repeat treatments. Despite huge advances in technology, most countries around the world are still using the same microscopic examination of sputum that was used over 100 years ago. With only 40 to 60% test sensitivity under field conditions, this falls as low as 20% when patients are co-infected with HIV. [3] Till today, especially in paucibacillary and extrapulmonary tuberculosis, the diagnosis is usually based on subjective evidence of anamnestic symptoms and varied clinical-radiological presentations. Without the right diagnostic tools, we cannot stop the TB epidemic. Developing new diagnostics is one of the six elements of the Global Plan to Stop TB: 2006 – 2015. [3] With this background, we took up our prospective study of the role of IGRA with QuantiFERON TB-Gold IN TUBE ASSAY, which is available in the Indian market, in the evaluation of suspected active tuberculosis, especially in a clinical setting where obtaining a confirmatory diagnosis is very unlikely.

Materials and Methods

This Prospective study was conducted in the Department of Pulmonology, Prathima Institute of Medical Sciences, Naganoor, Karimnagar. This study was approved by the Institutional Ethical Committee of the college. All the subjects were informed about the study procedure. Eligible subjects who were attending to pulmonology outpatient department in our hospital with symptoms and radiological features suspicious of tuberculosis and Fever of unknown origin (FUO) were enrolled. Well-informed consent was taken from the subjects in their native or local language.

Inclusion criteria

1. Suspected cases of active Tuberculosis. [4, 5]
2. Fever of unknown origin (classic).

Exclusion criteria

1. Smear positive pulmonary TB or Culture positive TB.
2. Histopathological examination suggestive of TB.
3. Individuals with a history of TB.
4. HIV infection, end-stage renal disease, Cirrhosis of the liver.

A total number of 53 subjects were included in the study, Complete blood picture, ESR, complete urine analysis, RBS, HIV, Serum creatinine, Blood urea, LFT were done. Wherever samples could be obtained, by suitable invasive and non-invasive methods, were subjected to histopathological examination, Bacterial and fungal cultures, and fluid analysis. All the subjects underwent a Tuberculin skin test and QuantiFERON TB Gold in Tube assay. All the patients where tuberculosis was strongly suspected based on clinico-radiological and other investigations, were started on anti-tuberculosis treatment.

Table 1- QuantiFeron TB Gold Assay interpretation.

Interpretation	Nil*	TB Response†	Mitogen Response§
Positive¶	≤8.0	≥0.35 IU/ml and ≥25% of Nil	Any
Negative**	≤8.0	<0.35 IU/ml or <25% of Nil	≥0.5
Indeterminate††	≤8.0	<0.35 IU/ml or <25% of Nil	<0.5
	>8.0	Any	Any

* The interferon gamma (IFN-γ) concentration in plasma from blood incubated without antigen.
† The IFN-γ concentration in plasma from blood stimulated with a single cocktail of peptides representing early secretory antigenic target-6 (ESAT-6), culture filtrate protein-10 (CFP-10), and part of TB 7.7 minus Nil.
§ The IFN-γ concentration in plasma from blood stimulated with mitogen minus Nil.
¶ Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.
** Interpretation indicating that *M. tuberculosis* infection is not likely.
†† Interpretation indicating an uncertain likelihood of *M. tuberculosis* infection.

All the patients were given category-I Anti-tuberculosis treatment under RNTCP guidelines and were regularly followed up, every 15 days for the first 2 months and every 1 month of the next four months. At follow up patients were evaluated for clinical improvement and adverse reactions. All the patients responded well with no deterioration and were kept on follow-up every two months after completion of treatment. Patients who were not diagnosed with tuberculosis were given appropriate treatment according to the diagnosis and were also kept on regular follow-up every two months. No patient

among non-tuberculous patients deteriorated or showed up any new symptoms.

Results

A total number of n=53 subjects were included in the study, of which males are n=27 (50.9%) and females are n=26 (49.1%) with an average age of 35 to 39 (range 4 to 71 years). Out of n=53 patients, 35 (66%) are from urban region and n=18 (34%) from rural region. The majority of patients were in the age group of 21 to 30 years n = 18, (34%). There were n=2 patients in the age group of fewer than 10 years (3.8%) and 6 elderly patients (10.5%). Most of the patients were from urban background (n = 35, 60%) and 18 (34%) were from rural background. There were 17% of illiterate (9 patients) with primary education of 3.8%, higher secondary in 7.5%. Patients who completed tenth standard were about 35.8% (n = 19) and graduates were 32.1% (n = 17). Socioeconomic status was assigned based on Kuppuswamy Scale [49]. Lower and upper-lower were 9 patients (17%) compared to lower-middle and above is 44 (83%), there were 7.5% (n = 4) in the upper socioeconomic status group. A low percentage of smokers was found in our study (n = 4, 7.6%). Almost all of the higher education group were nonsmokers (35/36) and in the other group, there were 14 out of 17 who were non-smokers. Almost similarly there were no chronic alcoholics in our study and 16 out of 53 (30.4%) were occasional alcoholics. There were more alcoholics 33.3% (even though occasional) in the higher education group (12 out 36).

Table 2: Demographic profile of the patients included in the study

Age in years	***Frequency (%)***
< 10	2 (3.8)
11- 20	8 (15.1)
21-30	18 (34)
31-40	4 (7.5)
41-50	8 (15.1)
51-60	7 (13.2)
61-70	5 (9.4)
> 71	1 (1.9)
Residence	***Frequency (%)***
Urban	35 (66)
Rural	18 (34)
Education	***Frequency (%)***
Illiterate	9 (17)
Primary	2 (3.8)
Middle	2 (3.8)
Higher secondary	4 (7.5)
Intermediate	19 (35.8)
UG / PG	17 (32.1)
Smoking	***Frequency (%)***
Yes	4 (7.5)
No	49 (92.5)
Alcohol	***Frequency (%)***
No	16 (30.1)
Occasional	37 (69.8)

The most common symptoms were cough (57%) and fever (55%). Among 29 patients with fever, 72.4% (n=21) were having evening rise of temperature, and night sweats were seen in 7.6% of all the patients and details have been depicted in table 3.

Table 3: Frequency of symptoms reported in the cases of study

Symptoms	Male		Female		Total
	(n)	%	(n)	%	N (%)
Cough	16	30.2%	14	26.4%	30 (56.6%)
Dyspnea	2	3.8%	5	9.6%	7 (13.2%)
Chest pain	5	9.4%	2	3.8%	7 (13.2%)
Fever	14	26.4%	15	28.3%	29 (54.7%)
Loss of appetite	6	11.3%	4	7.5%	10 (18.8%)
Loss of weight	6	11.3%	4	7.5%	10 (18.8%)

Presentation as Fever of unknown origin was noted in 20.8% of patients (n=11). Other symptoms included in patients with FUO were loss of weight in 4 patients (36%), cough in 6 patients (54.5%). 6 out of 53 patients had immunocompromised status with 4 patients being diabetic, 1 patient on oral corticosteroids, and one patient having carcinoma of the lung. Patient with malignancy had both QuantiFERON-TB Gold In-Tube test (QFT-GIT) and TST positivity and was not started on treatment with ATT. Chest x-ray was abnormal in 24 patients (45.6%) and 54.7% of patients have a normal x-ray. The most common abnormal finding was pleural effusion in 50% and consolidation in 45.8% and lymph node enlargement of the hilar region seen in one patient.

CT chest was done wherever indicated and one patient with suspected malignancy had associated pleural effusion. Erythrocyte sedimentation rate (ESR) after one hour was recorded in all patients; about 10 patients had less than 10 mm of ESR (19%). The majority showed an ESR in the range of 21 to 50 mm (n= 21, 40%) and only 19% (n = 10) had more than 50 mm of ESR recorded. So, most of the patients had an ESR of less than 50 mm (81.7%).

Table 4: Chest X-RAY findings VS QFT-GIT VS TST

Chest X-ray in Active TB	**TST**			
	Negative		positive	
	QFT-GIT		QFT-GIT	
	Negative	Positive	Negative	Positive
Normal	0	1	1	18
Consolidation	1	1	1	5
P.E.*	0	0	2	3
Hilar LAP**	0	0	0	1
Chest X-ray in Non-TB	**TST**			
	Negative		positive	
	QFT-GIT		QFT-GIT	
	Negative	Positive	Negative	Positive
Normal	7	0	1	1
Consolidation	1	0	2	0
P.E.*	1	0	3	1
Hilar LAP**	0	0	0	0

**One Pleural effusion (P.E) case was IGRA Indeterminate. ** Hilar lymphadenopathy*

The number of patients who had ESR of 50 mm or more with QFT-GIT positive was four (40%), and four were QFT-GIT negative with two indeterminates (20%). 16 out of 31 QFT-GIT positives were having ESR of less than 20 mm. TST positive had 7 patients out of 41 who had an ESR of more than 50 mm (17.03%) compared to 12.9% of QFT-GIT positives. The majority of QFT-GIT positive (87%) and TST positives (83%) had less than 50 mm ESR. Our study included patients with suspected Tuberculosis based on symptoms, but all the confirmative tests were not sent either due to absence of expectoration, pleural, peritoneal, or CSF (extra sanguineous fluids) involvement or due to the non-availability of tissue for cytological diagnosis or where these tests even when sent were not confirmative. N=11 out of n=53 patients had pulmonary parenchymal involvement (20.9%), 12 out of 53 (22.8%) had pleural involvement. The gastrointestinal disease was seen in 3 patients (5.7%), lymph nodal involvement was seen in 6 patients (11.4%), Musculoskeletal involvement in one case (1.9%), upper respiratory tract involved in 2 patients (3.8%), testicular involvement in 1 patient (1.9%), and ophthalmic involvement in 6 (11.4%) patients. In parenchymal disease with confirmed tuberculosis, the concordance of TST and QFT-GIT was 75% and discordance of 25%. In non-tuberculous parenchymal disease exclusion by both the tests (both are negative) in 1 out of 3 (33.3%) where TST was positive in 2 patients and QFT-GIT was negative in 2 patients

(66%). In tuberculous pleural disease concordance of TST and QFT-GIT was 3 out of 6 (50%) and discordance 2 out of 6 (33.3%) with 1 being of the indeterminate result. In excluding pleural disease of tubercular etiology concordance of QFT-GIT and TST was in 1 out of 5 (20%), whereas TST was positive in 4 patients (80%), and QFT-GIT in one case (20%) and in 80% patients QFT-GIT was negative in non-tubercular pleural effusions. (Ref table no. 5) In non-parenchymal, non-pleural extrapulmonary patients based on chest x-ray one patient had confirmed lymph node tuberculosis where TST and QFT-GIT were both positive. In patients with active tuberculosis (n = 35) TST was positive in 32 patients (91.4%), QFT-GIT was positive in 29 patients (82.8%) one case of indeterminate QFT-GIT was TST positive. Concordance between the two was 28 out of 35 (80%), discordance was 6 out of 35 (17.1%).

Table 5- sensitivity & specificity of all cases

Involved organ	TST			
	Negative		Positive	
	QFT-GIT		QFT-GIT	
	Negative	Positive	Negative	Positive
Active TB				
GIT	0	0	0	2
Lymph Node	0	0	0	6
Pleura	0	0	2	3
Lung parenchyma	1	1	1	5
URT	0	0	0	1
Eye	0	0	0	6
Non-TB	**Negative**	**Positive**	**Negative**	**Positive**
GIT	1	0	0	0
Pleura	1	0	3	1
Lung parenchyma	1	0	2	0
URT	0	0	0	1
Musculo skelet al	1	0	0	0
Testes	1	0	0	0

The overall Sensitivity, Specificity, PPV and NPV for QFT-GIT was 85.29%, 88.24%, 93.55% and 75% respectively similarly the TST 91.43%, 50%, 78.05% and 75%.

Discussion

Several novel methods were proposed to diagnose TB when confirmatory methods like direct smear microscopy and cultures were not feasible. Keeping the local epidemiological conditions in mind, clinical case definitions, based on symptoms and signs would help suspect active TB disease. [4, 6] This would further aid in the rigorous diagnostic workup. IGRAs, commercially available as the QuantiFERON-TB-Gold In-Tube, in India, are now established in many countries as a tool in the diagnosis of Latent TB Infection (LTBI). Its role in the diagnosis of active TB is being studied and reported with variable outcomes. [7-11] This may be at least partly due to differences in local epidemiological situations and populations studied, variation in the infecting strain, and inclusion of HIV-infected patients into the study. [7] Our study reports that both QFT-GIT and TST were highly sensitive in detecting active TB patients, with a poorer specificity for TST. The sensitivity of QFT-GIT was noted as 85.29% (CI 69.87 – 93.55) and that of TST as 91.43% (CI 77.62% - 97.04%). Sensitivity was further enhanced when both QFT-G and TST were combined. The combined sensitivity was thus 98.65%. Further statistical analysis of our study reveals a specificity of 88.24% for QFT-GIT (5 of the 20 QFT-G negative patients only treated) and poorer performance of 50% for the specificity of TST. Similarly, the Positive predictive values for QFT-GIT and TST were 93.55% and 78.05% respectively. Negative predictive values and Accuracy were 75% and 86.27% respectively for both the tests. Our results, like that of Yoshihiro Kobashi et al., [11] also confirmed that the higher specificity of QFT-G supported the usefulness of the test in BCG-vaccinated individuals.-Though QFT-G may not be used for excluding active TB, its high negative predictive value is an important indicator for a TB control program therefore it may limit therapy to contact people with a positive test. [12] Ling et al., [8] in their study observed the sensitivity of QFT-GIT regarding smear-negative patients was 75% and specificity 37%. The lower results were ascribed to the high burden settings and they inferred that QFT may not be useful as rule in or out active disease. One study from India [7] and the other from South Africa [13] reported higher sensitivity of 91% and 76% respectively and the combined sensitivity of QFT and TST being more than 96% in both studies. Similar results were obtained by Daniel Bendayan et al., [14] who noted QFT positive in 53% of patients.

The lower sensitivity in their study was explained by the fact that interferon secretion is usually diminished during the early stage of the disease and to the decreased immunological defense of the study population (immunocompromised state, elderly). The urgent need for alternative diagnostic assays is emphasized by the fact that more than 50% of pulmonary TB cases and most of all TB suspects have undetectable AFB in sputum smear examination and more than 10% of secondary TB cases are contracted from persons with negative AFB sputum smear. [15] In our study higher sensitivity for QFT (in a high burden setting) is attributable to exclusion of HIV individuals, in contrast to earlier studies which included HIV patients, the possible effects of genetic makeup in the population, and the variation in the infecting M. tuberculosis strains may also be alternative explanations for the higher sensitivity for QFT-GIT in the present study and earlier reports. [7] Lee et al., [16] suggested that using lower cut-off IFN-γ values for positive QFT-G results may increase the sensitivity of the assay with the minimal trade-off in specificity. This should be considered with a high pre-test probability of active tuberculosis. KA Kanunfre et al., [17] defined interferon-γ levels of > 0.20 IU/ml as being the cut-off level for the diagnosis of TB in their study, resulting in a sensitivity of 86% (CI 73.6% - 98.7%) and a specificity of 100%. Thus, a lower cut-off for QFT-GIT for active TB (in comparison to LTBI) to be recommended after further larger studies in high burden countries like India; varied results of sensitivities in previous studies were found to be highly dependent on the study population, notably local- TB prevalence. [18]

Conclusion

In conclusion, a positive QFT-GIT result improved clinical evaluation of tuberculosis suspects, especially in patients where a diagnostic confirmation was not possible, viz., extrapulmonary TB, and FUO. IGRAs are superior to the TST because they are unlikely to be falsely positive. Further studies setting different cut-off values for active TB and LTBI and lower cut-off values for IGRAs in high burden countries may show realistic values for this assay in the diagnosis of active TB.

***Conflict of Interest*: None**
***Source of support*: Nil**
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