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Accuracy of QuantiFERON-TB Gold plus Test for Diagnosis of Mycobacterium tuberculosis Infection in various age group individuals in Nalgonda district.

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Aim and objective of the study: To evaluate accuracy of QuantiFERON-TB Gold Plus (QFT-Plus) in showing sensitivity against active tuberculosis (TB) or latent TB infection (LTBI). QFT-Plus contains an additional antigen tube (TB2), stimulating both CD4 + and CD8 + T cells. The ability to discriminate CD4 + and CD8 + responses is suggested to be useful in differentiating stages of Mycobacterium tuberculosis infection.

Materials and methods: A prospective cross-sectional study was conducted among individuals aged 17-65 years who were evaluated for suspected active TB or LTBI. All individuals underwent QFT-Plus and further clinical, radiological, and/or microbiological analyses according to clinical scenarios. Of the 90 individuals enrolled from various Rural Primary Health care centres (PHCs) at Nalgonda district i.e Narketpally (10 individuals), Line wada (12 individuals), Damercherla (12 individuals), Manyamchelka (16 individuals), Bangarugadda (12 individuals), Ramulavada (16 individuals), Prakash nagar (12 individuals).

Results: All individuals underwent QFT-Plus, out of which 70 (77.77%) were tested because of suspicion of active TB. A total of 30/70 (42.85%) were diagnosed with active TB, and among these, 28/30 (93.3%) had a positive QFT-Plus assay. Of the 20 individuals screened for LTBI, 12 (60%) had a positive QFT-Plus, and 02 (10.0%) had an indeterminate result. TB1 and TB2 quantitative responses were calculated.

Conclusions: QFT-Plus assay is accurate in showing good sensitivity for active TB (particularly pulmonary TB). QFT-Plus can be particularly useful for the evaluation of individuals with suspected LTBI, giving a very low rate of indeterminate results in this group.

Keywords: QuantiFERON-TB Gold Plus Test, Interferon Gamma Release Assay(IGRA), Active tuberculosis (TB), Latent TB infection (LTBI), Mycobacterium tuberculosis

I. INTRODUCTION:

Worldwide, tuberculosis (TB) is a major public health threat, causing an estimated 8.6 million new cases and 1.3 million deaths from TB in 2012. Initially, TB infection is eliminated or constrained by the host's immune system, and infection remains latent. Active is symptomatic TB. Latent TB is asymptomatic and non-infectious. However, latent TB bacilli may remain viable and "reactivate" later to cause active TB disease. Early identification and treatment of LTBI can eventually reduce the risk of development of disease and are important TB control strategies, especially in settings with a low TB incidence, where reactivation of LTBI often accounts for the majority of non imported TB disease.

TB disease is commonly diagnosed by a positive Tuberculin Skin Test (TST) result, epidemiological information (exposure to a known source case) and through clinical and radiographic presentation. For decades, the TST was the only test available for diagnosing latent TB infection (LTBI), though both false-negative and false-positive results plague this old assay.

Recently, interferon- release assays (IGRAs) have been developed to replace the TST for detecting LTBI. The sensitivity and specificity of the TST and IGRAs were almost similar for diagnosing active TB, while it remains difficult to determine the accuracy in detecting LTBI, given the lack of gold standards in children and adults. The TST has several known limitations like false-positive and false-negative results can occur. There are two important causes of false-positive results: nontuberculous mycobacterium (NTM) infection and prior BCG vaccination. False-negative TST results may occur because of limited sensitivity in particular patient subgroups (e.g., immunocompromised individuals [due to HIV

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infection, cancers or malnutrition] or those taking immunosuppressive medications, organ transplant patients) or because of preanalytical or analytical sources of test variability (e.g., improper tuberculin handling or placement or incorrect interpretation of test results).

Fortunately, the advantage of QuantiFERON-TB Gold-In-Tube (QFT-IT) (as well as other IGRAs) rests on its higher specificity in BCG-vaccinated subjects, preventing unnecessary and potentially toxic treatments.

IGRA	TST
One Visit	Two visits (4 for two - step test)
No cross reaction with BCG	Cross reacts with BCG with potential for false positives
Limited time frame from draw to incubation in lab	Times frame constraint for reading
More expensive than TST	Less expensive than IGRA
Less subjective determination of results	More subjective determination of results
Not approved for children under age 5 years	Unreliable results for children under age 6 months

Recently, the 4th-generation QuantiFERON-TB Gold Plus (QFT-Plus) IGRA has been launched. It includes two tubes rather than one tube containing peptides of the M. tuberculosis-specific antigens EsxA and EsxB as follows: TB1, which stimulates mainly CD4 + T cell responses; and TB2, which stimulates both CD4 + and CD8 + T cells. TB1 contains comparatively long synthetic peptide cocktails to mainly stimulate CD4+ T cells, whereas TB2 also contains short peptide cocktails to stimulate both CD4+ and CD8+ T cells. IFN-γ-producing CD8 T cells specific for these two M. tuberculosis antigens are more frequently detected in active TB patients than they are in subjects with LTBI.

In this scenario, we performed this study aiming to evaluate the accuracy of the QFT-Plus assay in individuals belonging to various age groups in Nalgonda district with suspected active TB and LTBI.

II. MATERIALS AND METHODS:

A prospective cross-sectional study was conducted among individuals aged 17-65 years who were evaluated for suspected active TB or LTBI. All individuals underwent QFT-Plus and further clinical, radiological, and/or microbiological analyses according to clinical scenarios. Of the 90 individuals enrolled from various Rural Primary Health care centres (PHCs) at Nalgonda district i.e Narketpally (10 individuals), Line wada (12 individuals), Damercherla (12 individuals), Manyamchelka (16 individuals), Bangarugadda (12 individuals), Ramulavada (16 individuals), Prakash nagar (12 individuals).

QuantiFERON-TB Gold Plus: QFT-Plus was performed according to manufacturer's instructions. Data are presented as IU per millilitre of IFN-γ; the cutoff value for a positive test was 0.35 IU/ml.

Step by step procedure of QuantiFERON-TB Gold Plus:

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PROCEDURE		
DAY-1	SAMPLE COLLECTION	3x QFT blood tubes containing nol control, Mtb antigens (comgined) or mitogen control ●1ml blood per tube ●Total blood vol = 3ml
	PRE INCUBATION	Inversion of tubes to ensure mixing of antigens /controls with blood Mtb antigens ESAT-6, CFP-10 TB 7.7
	INCUBATION	16-24 hour incubation in QFT blood tubes (37° C)
DAY-2	POST - INCUBATION	Centrifugation of QFT blood tubes for separation of plasma
	ASSAY PROCEDURE	
AFTER DAY -2	INTERPRETATION	ELISA for quantification of plasma IFNy levels Result determination using ELISA reader

All patients were clinically assessed and tested by QFT-Plus. All individuals with a positive QFT-Plus or high clinical suspicion of TB disease underwent radiographic and microbiological investigations to confirm or rule out active TB. Microbiological diagnosis included acid-fast bacilli (AFB) examination following Ziehl-Neelsen staining, culture for M. tuberculosis, and molecular detection of M. tuberculosis using the Anyplex MTB/NTM real-time detection system (Seegene) following previously indicated procedures. The samples were obtained on three consecutive days (either sputum or gastric gavage according to age) from all patients with suspected active TB. For those with suspected extrapulmonary TB, samples were taken from different sites according to the suspected TB localization. All individuals with a final diagnosis of active TB were evaluated for HIV infection. Two definitions of active TB were used as follows: definite (confirmed) and probable TB, as accepted by literature regarding TB.

LTBI was diagnosed based on positive QFT-Plus and absence of any clinical, microbiological, and radiographic features that suggest it is active TB. Hence, according to the definitions used, the following final diagnoses were assigned for each patient at the end of clinical evaluations: active TB, LTBI, or non-TB

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individuals (no active TB or LTBI). This latter group included both healthy/asymptomatic individuals without LTBI (healthy) and all individuals with any other final diagnosis other than TB, such as bacterial, viral, fungal, or parasitic infections (disease other than TB).

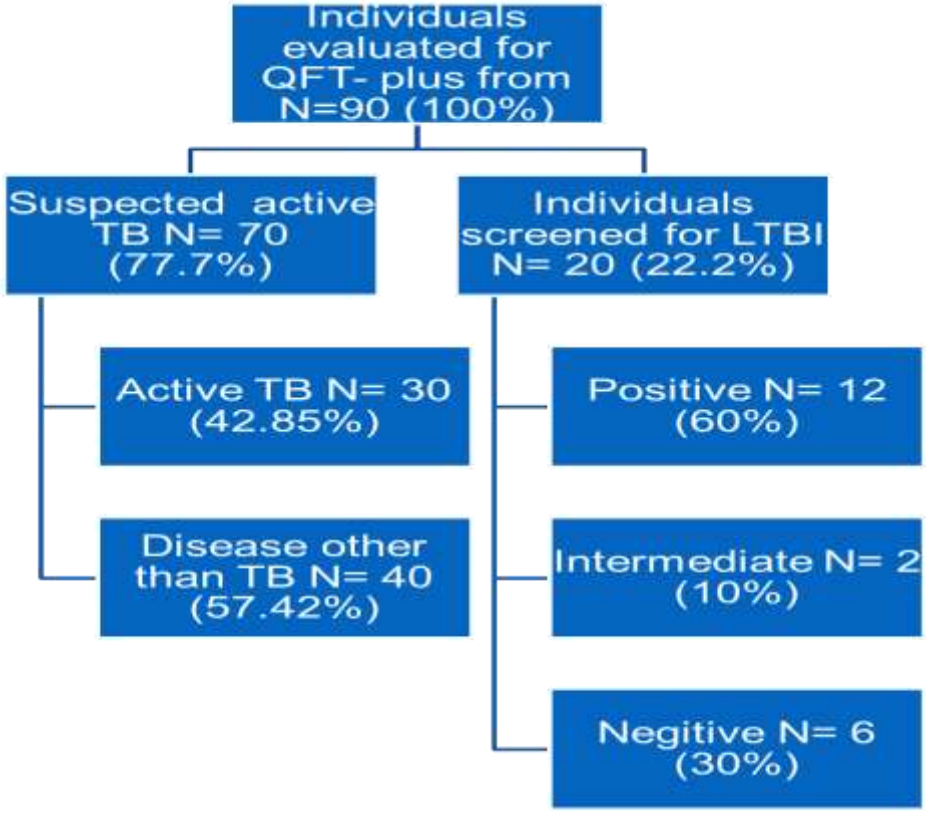
Statistical analysis: Differences in frequencies were evaluated by the Fisher exact test. The median IFN-γ production was calculated.

Ethical consideration: The Declaration of Helsinki guidelines must be followed. The subjects gave informed consent. Patient anonymity has been preserved.

III. RESULTS:

Of the 90 individuals enrolled from various Rural Primary Health care centres (PHCs) at Nalgonda district i.e Narketpally (10 individuals), Line wada (12 individuals), Damercherla (12 individuals), Manyamchelka (16 individuals), Bangarugadda (12 individuals), Ramulavada (16 individuals), Prakash nagar (12 individuals).

All individuals underwent QFT-Plus, out of which 70 (77.77%) were tested because of suspicion of active TB. A total of 30/70 (42.85%) were diagnosed with active TB, and among these, 28/30 (93.3%) had a positive QFT-Plus assay. Of the 20 individuals screened for LTBI, 12 (60%) had a positive QFT-Plus, and 02 (10.0%) had an indeterminate result. TB1 and TB2 quantitative responses were calculated.



Measurement of the IFN-γ secretion in response to mitogen in IGRAs provides a precious indication of the potential ability of the immune system to respond to the antigenic stimuli. A total of 89/90 (98.88%) individuals of various age groups responded to the mitogen by secreting high levels of IFN-γ. Overall, only 2/90 had an indeterminate result, which indicates subjects unable to properly respond to the mitogen.

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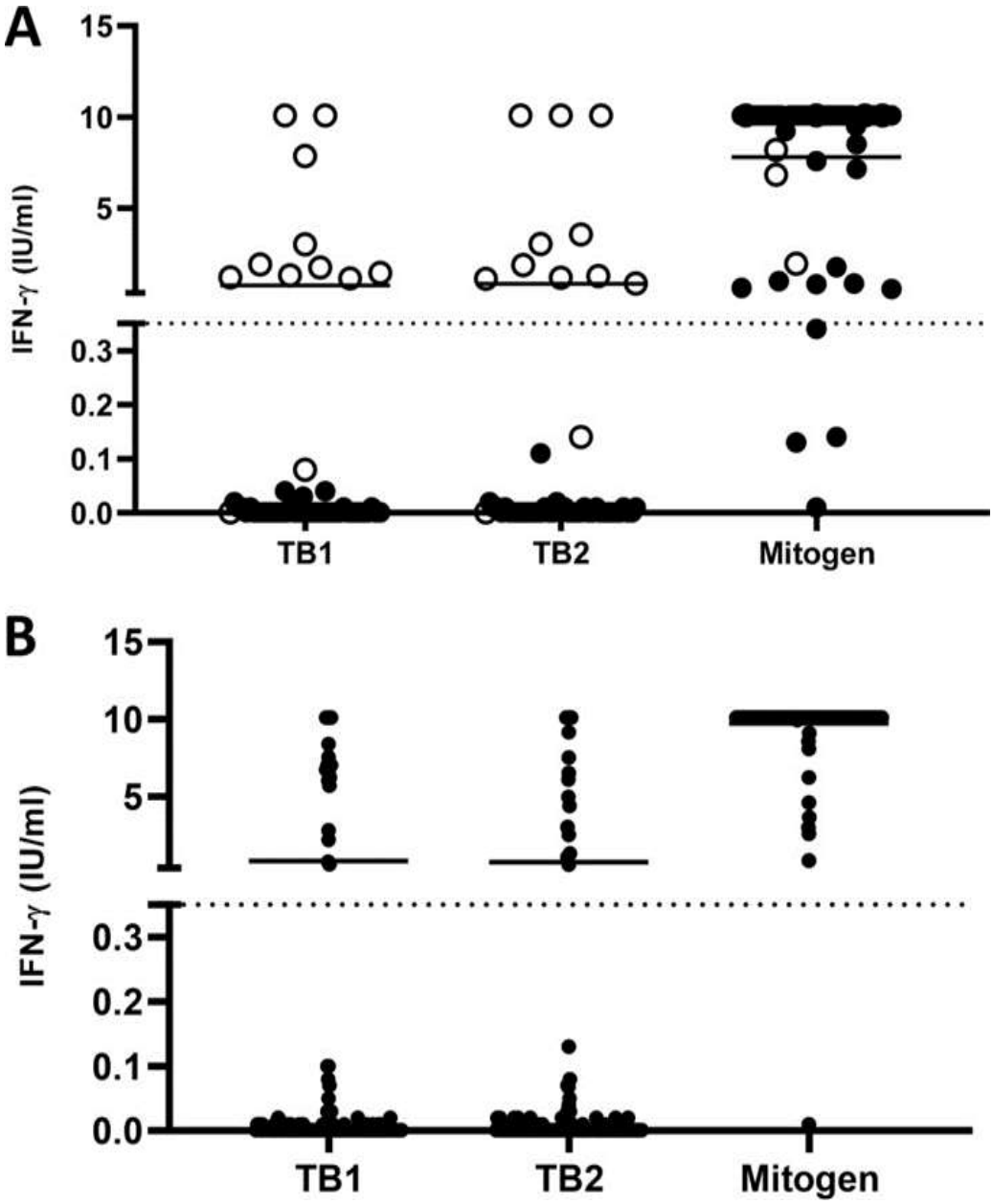

(A) IFN-γ response to TB1 and TB2 antigens in individuals with suspected active TB. Each point on the graph represents a separate result. Horizontal lines indicate the median of IFN-γ production. Dotted lines indicate the cutoff for QFT-Plus (0.35 IU/ml).

(B) IFN-γ response to TB1 and TB2 antigens in individuals screened for LTBI. IFN-γ was determined using enzyme-linked immunosorbent assay (ELISA) following whole-blood stimulation with QFT-Plus antigens for 24 h. Each point on the graph represents a seperate result. Horizontal lines indicate the median of IFN-γ production. Dotted lines indicate the cutoff for QFT-Plus (0.35 IU/ml).

Among the 2 individuals with indeterminate results, one had congenital immunodeficiency and one individual had autoimmune disorder (SLE). There was no statistically significant effect of age or of the final diagnosis on the magnitude of the response to mitogen. Only 2/20 (10%) asymptomatic children screened for LTBI had indeterminate results.

Although LTBI individuals had slightly higher mean values of TB1 responses over TB2 and active TB individuals showed the opposite pattern, we could not discriminate between active TB from LTBI. Overall, the IFN-γ responses were not significantly affected in various age groups.

IV. CONCLUSIONS:

QFT-Plus assay is accurate in showing good sensitivity for active TB (particularly pulmonary TB). QFT-Plus can be particularly useful for the evaluation of individuals with suspected LTBI, giving a very low rate of indeterminate results in this group.

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