Research Article

Interferon Gamma Release Assays (IGRA) in the Diagnosis of Active Pulmonary Tuberculosis

Mona K. EL-Deeb [1 ¥], Hatem A. EL Malwany [2], Yehia M. Khalil [2], Saher T. Mourad [2]

[1] Chemical Pathology Department, Medical Research Institute, Alexandria University, Egypt
[2] Chest Diseases Department, Faculty of Medicine, Alexandria University, Egypt

ABSTRACT

Background: Tuberculosis is one of the deadliest diseases over the world. Diagnosis of pulmonary TB depends on combination various parameters. IGRA measure T cell release of IFN-γ in response to M.TB antigen. WHO policy statement as on IGRA use in low and middle income countries is not established yet. **Objective**: The present study aimed to compare between tuberculin skin test (TST) and interferon gamma release assay (IGRA) in the diagnosis of active tuberculosis infection and study the effect of 3 months of first line anti-TB therapy on the positivity of the IGRA test.
Methods: 40 Egyptian patients were included in the study, and assigned as two groups; Group I comprised 20 patients with negative sputum for AFB by Ziehl-Neelsen stains with positive sputum culture for M.TB and Group II included 20 patients with positive sputum and Ziehl-Neelsen for AFB before and after 3 months of first line of anti-TB therapy. All patients were subjected to full history taking, clinical examination, X- ray chest, lab investigations, ESR measurements, microbiological tests and ELISA measurement of Quantiferon-TB Gold.
Results: Lower significant values were found in group II after treatment than before treatment regarding clinical parameters and 1st and 2nd hours ESR. IGRA test and TST showed sensitivity (91.18%, 76.4%), specificity (83.33%, 66.67%), positive predictive value (96.88%, 92.86%), negative predictive value (62.5%, 33.3%) and accuracy of (90%, 75%) respectively. IGRA results had no statistical significant differences between the studied groups with poor agreement with TST ((κ) = 0.025).
Conclusion: IGRAS test had high sensitivity and specificity in diagnosis of active TB. More studies are needed to evaluate the effect of anti-TB therapy on IGRA level.

Key words: *Interferon gamma release assays (IGRA), tuberculosis (TB), tuberculin skin test (TST)*

Available on line at:
www.ebscohost.com

¥*Correspondence*:
Email:mona_moh_eldeeb@yahoo.com

Tel: 002 01001993114

Suggested Citation: EL-Deeb MK,, Malwany HA, Khalil YM, Mourad ST. Interferon Gamma Release Assays (IGRA) in the diagnosis of active pulmonary tuberculosis. Bull. HIPH.2014;44(1):33-40.

INTRODUCTION

Tuberculosis (TB) is a chronic bacterial infection caused by the Mycobacterium tuberculosis class of bacteria. [(1)] It is one of the deadliest diseases in the world and TB infection has a yearly increase of over 9.4 million, with ninety five percent of these cases occurring in developing countries.[(2,3)] Alveolar macrophages play an important role in the formation of tuberculous granuloma.[(4)] Macrophages produce cytokines including interleukin-1(IL-1), IL-6, IL-10, tumour necrosis factor alpha (TNF- α) and transforming growth factor beta (TGF-β), which have the potential to exert potent immunoregulatory effects and mediate many of the clinical manifestations of tuberculosis , these cytokines are also elevated in many other disease such as: viral or bacterial pneumonia and brucellosis.[(5)] T lymphocytes also produce chemotactic cytokines, macrophage activating factors such as IFN γ, TNF- α, and IL -2. T-helper cells (Th1 and Th2) also can activate macrophage to inhibit or destroy ingested tubercle bacilli.[(6)]

Diagnosis of pulmonary tuberculosis depends on clinical suspicion, staining for acid fast bacilli (AFB), culture for mycobacteria, chest radiograph and response to treatment,. Other methods also aid in the diagnosis of pulmonary TB which include: nucleic acid amplification (NAA) assays, high performance liquid chromatography (HPLC), Polymerase chain reaction (PCR) and others. [(7)] The diagnosis of pulmonary TB must be done on combination of clinical, epidemiological parameters and imaging studies due to high false negative results of all microbiological method for TB diagnosis (10% to 15%) and the non

tuberculous mycobacteria which may be present in smears.[8] The anti-tuberculosis treatment has two main objectives. Firstly, rapid killing of bacilli living extracellular in lung cavities. Secondly, achieve complete elimination of those bacilli replicating less actively in acidic and anoxic closed lesions, and to kill semi dormant bacilli living intracellular in other host tissues, in order to avoid subsequent TB relapses. Thus combination therapy is preferred. Treatment modalities include drugs approved by the food and drug administration (FDA) eg: isoniazid (INH), pyrazinamide (PZA), rifampin (RIF) and rifapentine (RPT).[9] In addition to Immunotherapy and inhaled aerosolized IFN-γ.[10] Interferon-gamma release assays (IGRA) are in vitro blood tests of cell-mediated immune response; they measure T cell release of interferon-gamma (IFN-γ) following stimulation by antigens unique to M. tuberculosis.[10] The first commercially available test was QuantiFERON TB assay; which measures IFN γ production by ELISA after in vitro stimulation of white blood cells with PPD.[11] New generation of tests has been developed. They are the QuantiFERON-TB Gold (QFN-Gold) tests and the ELISPOT assay of TB (T SPOT-TB) test, which incorporate highly specific M. tuberculosis targets. IGRA are now being incorporated into national guidance for diagnosis and research is ongoing into next-generation versions of the test.[12]

Evidence suggests that both tuberculin skin test (TST) and IGRA are acceptable but imperfect tests in diagnosis of latent TB infection (LTBI).[13] However IGRA can be of help in testing for latent tuberculosis infection when used to identify individuals who are at increased risk for the development of tuberculosis and therefore who would benefit from treatment of latent TB infection as most individuals with a positive IGRA do not progress to TB disease.[14] In addition T-SPOT.TB has high sensitivity which may be useful for evaluating individuals with immunosuppressive conditions.[15]

IGRA are preferred over tuberculin skin test (TST) in certain conditions which include individuals who have received BCG (either as a vaccine or for cancer therapy) and individuals who have historically poor rates of return for TST reading , while TST is preferred over IGRA for testing children less than 5 years of age according to the Centers for Disease Control (CDC) 2010 guidelines.[16]

The World Health Organization (WHO) policy statement on IGRA for use in low and middle income countries is not established yet, however available evidence suggests that tuberculin skin test (TST) and IGRA have similar accuracy and performance in low and middle income countries as in developed settings, and there is no evidence that IGRA is superior to TST in these settings. [11,13,17]

The present study thus aimed to add to the available evidence and compare between interferon gamma release assay (IGRA) and tuberculin skin test (TST) in the diagnosis of active tuberculosis (TB) infection, also study the effect of 3 months(to give maximum chance for T cell activation) of the first line of anti-TB therapy on the positivity of the IGRA test in a group of Egyptian patients with active pulmonary tuberculosis.

METHODS

Study setting & design

We conducted a case-control study on 40 patients with pulmonary tuberculosis their age ranged from 20-40 years (12 females and 28 males (30% and 70% of cases respectively). Patients were selected from EL Maamora Hospital. On the basis of sputum smears for acid fast bacilli patients were divided into 2 main groups designated Group I (patient control group) that comprised 20 patients with negative sputum for AFB by Ziehl-Neelsen stain but have positive sputum culture (Löwenstein-Jensen) for Mycobacterium tuberculosis and Group II (IIa and IIb) that comprised 20 patients with positive sputum for AFB by culture and Ziehl-Neelsen stains was assessed before and after 3 months of anti-TB therapy using the first line drugs. Subjects with HIV, viral or bacterial pneumonia and brucellosis were excluded from the study. Subjects on immunosuppressant treatment or had immunosuppressive diseases (lymphoma, leukemia and other malignancies) were also excluded.

Clinical examination

History of the present illness was taken from all patients with symptoms of tuberculosis including: productive cough, hemoptysis, breathlessness, weight loss, anorexia, malaise, fever and night sweating. All patients were subjected to complete clinical examination with special focus on chest examination, radiological examination using standard postero-anterior chest radiographs and CT scan.

Laboratory Investigations

A diagnostic work up for all patients was followed that included; complete blood picture, [18] measurement *of* liver enzymes including serum levels of alanine and aspartate aminotransferases (ALT and AST respectively), [19] assessment of renal function tests: urea and creatinine serum levels, [20] assessment of fasting blood glucose level,[21] and erythrocyte sedimentation rate (ESR) after first and second hours. For Mantoux tuberculin skin test, 0.1 ml purified protein derivative (PPD) containing 5 tuberculin units was injected intradermally at the mid volar aspect of the left forearm. Reading of the test was done after 48 hours by measuring maximum transverse diameter of the skin induration (palpable, raised, hardened area or swelling) during this

period. Skin induration of 10 millimeters or more was considered positive.[22] Sputum smears for acid-fast bacilli were done for all patients by obtaining early morning expectorated sputum specimens after a deep productive cough on three consecutive days from each patient in a clean tightly closed plastic disposable container properly labeled and stained with Ziehl–Neelsin stain. The specimens were spread and fixed over a microscope slides, stained with basic fuchsin in ethanol as a primary stain and decolorized with acid-alcohol solution. The slides were examined under the microscope for the detection of AFB.[23] Acid fast bacilli were quantified as follows: (1+) 1–9 AFB/100 fields, (2+) 1–9 AFB/10 fields, (3+) 1–9 AFB/field, (4+) >9 AFB/field. Sputum smears were repeated in group IIb(after 3 months of anti-TB therapy) and results were compared with results of group IIa. Sputum cultures of mycobacteria using Löwenstein–Jensen cultures were also done to all patients.[24] Sputum culture were repeated in group IIb (after 3 months of anti-TB therapy) and results were compared with results of group IIa. For the measurement of interferon gamma release using QuantiFERON-TB Gold [QFT-IT] [25], one ml of whole blood samples were collected into each of the Quanti FERON-TB Gold blood collection tubes (Nil control tube and TB antigen Tube were used for each case).Tubes were incubated at 37^{o}C for 16- 24 hours, centrifuged for 15 minutes at 2000 to 3000 RCF then the plasma was collected after gel separation and stored for 4 weeks at 8^{o}C.Interferon Gamma (IFN γ) production was measured after in vitro stimulation of white blood cells in response to peptide specific antigens. The assay was done using ELISA technique following manufacturer instructions where positive cases considered if the nil tube value is ≤ 8.0 IU/ml and the difference between the value of TB antigen tube and the nil tube is ≥ 0.35 and ≥ 25% of the nil value.

Statistical analysis of the data [26]: Statistical analysis was done using IBM SPSS software package version 20.0. Qualitative data were described using number and percent and their comparison was tested using Chi-square test. Correction for chi-square was conducted using Firsher's Exact test or Monte Carlo correction when more than 20% of the cells have expected count less than 5. Quantitative variables were tested for normality. For normally distributed data, comparison between two independent population were done using independent t-test and paired t-test was to used to analyse two paired data. For abnormally distributed data, Mann-Whitney Test was used to analyze two independent population. Wilcoxon test was used to compare between the different periods. Agreement between Tuberculin test and Quantiferon TB gold test was assessed by Kappa test and comparison between their predictive outcomes was expressed in terms of sensitivity, specificity, positive predictive value, negative predictive value and accuracy.

Ethical Statement

The study was approved by the institutional review board and the ethics committee of the Faculty of Medicine affiliated to Alexandria University, Egypt. The research complied with the international ethical research guidelines of declaration of Helsinki. All participants were invited to sign an informed written consent after explaining the aim and concerns of the study. Data sheets were coded to ensure anonymity and confidentiality of patient's data.

RESULTS

Table (1) shows the clinical data of the studied groups. Lower statistical significant difference was found regarding chest pain ,cough , weight loss and absence of low grade fever in group II after treatment than before treatment ($p < 0.001$, $p = 0.003$, $p < 0.001$, $p < 0.001$respectively) with significant decrease in night sweating ($p_1 < 0.001$ and $p_2 = 0.003$) in group II after treatment than both group I and group II before treatment . Which indicate good clinical response to treatment.

Table 1: Comparison between the studied groups according to clinical data

	Group I (Negative AFB by Zeihl-Neelsen stain but positive sputum culture)	**Group II (Positive AFB by Zeihl-Neelsen stain before treatment)**	**Group II (Positive AFB by Zeihl-Neelsen stain after treatment)**
Dyspnea			
p_1		MCp = 1.000	MCp = 0.732
p_2			^{WRST}p = 0.109
Chest pain			
p_1		MCp = 0.407	MCp<0.001*
p_2			p <0.001*
Cough			

p_1	FEp = 0.487	FEp = 0.003*
p_2		p = 0.055
Expectoration		
p_1	FE = 0.049*	□□p = 0.197
p_2		p = 0.250
Hemoptysis		
p_1	□□p = 0.919	□□p = 0.098
p_2		p = 0.082
WT loss		
p_1	FEp = 1.000	FEp<0.001*
p_2		p <0.001*
Low grade fever		
p_1	FEp = 0.231	FEp<0.001*
p_2		p <0.001*
Night sweating		
p_1	FEp = 0.020*	FEp<0.001*
p_2		p = 0.003*

MCp: p value for Monte Carlo test FEp: p value for Fisher Exact test
p: p value for McNemar test χ^2p: p value for Chi-square test
$_{WRST}$p: p value for Wilcoxon signed ranks test
p_1: p value between group I and group II before and after treatment
p_2: p value between group II before and after treatment
*: Statistically significant at $p \leq 0.05$

Table 2: Comparison between the studied groups according to the erythrocyte sedimentation rate (1st and 2nd hours)

	Group I (Negative AFB by Zeihl-Neelsen stain but positive sputum culture)	Group II (Positive AFB by Zeihl-Neelsen stain before treatment)	Group II b(Positive AFB by Zeihl-Neelsen stain after treatment)
ESR 1st hour mm(<10)			
Min. – Max.	77.0 – 117.0	70.0 – 120.0	40.0 – 109.0
Median	99.50	97.50	90.50
p_1		0.719	0.006*
p_2			0.049*
ESR 2nd hour mm(<20)			
Min. – Max.	101.0 – 151.0	122 – 155.0	60.0 – 154.0
Median	143.0	142.50	128.0
p_1		0.719	0.003*
p_2			0.003*

p_1: p value between group I and groupII before and after treatment
p_2:p value between group II patients before and after treatment

Table 3: Comparison between the studied groups according to Tuberculin test, Sputum AFB and sputum culture

	Group I (Negative AFB by Zeihl-Neelsen stain but positive sputum culture)		Group II (Positive AFB by Zeihl-Neelsen stain before treatment)		Group II (Positive AFB by Zeihl-Neelsen stain after treatment)	
	No.	**%**	**No.**	**%**	**No.**	**%**
Tuberculin test						
Positive (out of total 20)	12	60.0	16	80.0	16	80.0
p_1			FEp = 0.301		FEp = 0.301	
p_2					p = 0.115	
Sputum AFB						
Negative	20	100.0*	0	0.0	10	50.0*
+ (1 – 9 AFB/100 field)	0	0.0	4	20.0	6	30.0
+ + (1 – 9 AFB/ 10 field)	0	0.0	3	15.0	1	5.0
+ + + (1 – 9 AFB/ field)	0	0.0	10	50.0	3	15.0
+ + + + (> 9 AFB/ field)	0	0.0	3	15.0	0	0.0
p_1			MCp<0.001*		MCp = 0.001*	
p_2					$_{WRST}$p = 0.002*	
Culture						
Positive	20	100.0	14	70.0	14	70.0

Negative	0	0.0	6	30.0	6	30.0
p_1			FEp = 0.020*		FEp = 0.020*	
p_2					p = 0.115	

Comparison between studied groups as regard Erthyrocyte sedimentation rate (ESR) where shown in table (2). First hour ESR values were significantly higher in group I and group II before treatment than group II after treatment (p= 0.006, p=0.049) respectively and second hour values were also significantly higher in group I and group II before treatment than group II after treatment (p=0.003 ,p=0.003)indicating good clinical response. Negative sputum for AFB results in groupII after treatment was statistically higher than before treatment . (table3) indicating good response to treatment. Table (4) showed comparison between IGRA and tuberculin skin test regarding sensitivity, specificity, positive and negative predictive values where IGRA had sensitivity (91.18%) , specificity (83.33%) , positive predictive value (PPV) (96.88%) and negative predictive value (NPV) (62.5%) and accuracy of (90%) while Tuberculin test had sensitivity (76.47%), Specificity (66.67%), positive predictive value (PPV) (92.86%) and negative predictive value (NPV) (33.3%) and accuracy of (75%). No statistical significant differences was found between the three studied groups regarding Quantiferon TB gold test (table5). Agreement between Tuberculin test and QFT-Gold IT test was shown in (table 6), where the Kappa (κ) was 0.025 which indicate poor agreement.

Table 4: Comparison between Quantiferon TB gold test and tuberculin skin test

		Culture		Sensitivity	Specificity	PPV	NPV	Accuracy
		-ve	+ve					
Quantiferon TB gold test	**-ve**	5(TN)	3(FP)	91.18	83.33	96.88	62.50	90.0
	+ve	1(FN)	31 (TP)					
Tuberculin	**-ve**	4(TN)	8(FP)	76.47	66.67	92.86	33.3	75.0
	+ve	2 (FN)	26 (TP)					

Table 5: Comparison between the studied groups according to Quantiferon TB gold test

	Group I (Negative AFB by Zeihl-Neeelsen stain but positive sputum culture)		Group II (Positive AFB by Zeihl-Neeelsen stain before treatment)		Group II (Positive AFB by Zeihl-Neeelsen stain after treatment)	
	No.	**%**	**No.**	**%**	**No.**	**%**
Quantiferon TB gold						
Negative	3	15.0	5	25.0	4	20.0
Positive	17	85.0	15	75.0	16	80.0
p_1			FEp = 0.695		FEp = 1.000	
p_2					p = 0.246	
TB Antigen						
Min. – Max.	0.0 – 9.65		0.0 – 9.97		0.26 – 1526.0	
Mean ± SD	5.36 ± 4.07		4.49 ± 3.85		79.27 ± 340.54	
Median	6.60		3.66		2.32	
p_1			^{t}p = 0.705		^{t}p = 0.402	
p_2					p = 0.502	

Table 6: Agreement between Tuberculin test and QFT-Gold IT

	Quantiferon TB gold test				Kappa
	Negative		Positive		
	No.	**%**	**No.**	**%**	
Tuberculin test					
-ve (12)	5	62.5	7	21.9	0.025
+ve (28)	3	37.5	25	78.1	
	8	100	32	100	

*: Statistically significant at $p \leq 0.05$

DISCUSSION

Over nine million new cases of tuberculosis and two million deaths from this disease occur yearly worldwide.[3] Thus early diagnosis and prompt treatment of patients with active pulmonary disease are the most important factors in reducing the morbidity, mortality, and incidence of TB.[27] Limitations of the traditional microbiological methods have led to the development of additional methods, such as the gamma interferon (IFN- γ) release assay, as an aid in clinical and laboratory investigations of cases of human TB. [28] However, IGRA were explicitly designed to replace the TST in diagnosis of latent taberculous infection and were not intended for active tuberculosis, which is a microbiological diagnosis.[29] In most low- and middle-income countries, these tests can be expected to have poor specificity for active tuberculosis in all high-burden settings because of a high background prevalence of LTBI.[30] In addition malnutrition, human immunodeficiency virus (HIV)–associated immune suppression and laboratory procedures and infrastructure, may also contribute to a lower performance of IGRA observed in these countries.[31] .However, private sector laboratories in high burden countries,[32] and many investigators continue to recommend the use of IGRA for active tuberculosis diagnosis.[32.33] When comparing the studied groups regarding signs and symptoms, persistence of signs and symptoms in groupI(negative sputum for AFB by Ziehl-Neelsen stains but positive sputum culture) may be due to the permanent damage that goes with healing.[34] Statistical significantly lower results in group II (after therapy) regarding signs and symptoms (table1)are in accordance with Lee et al [35] who concluded that all patients had clinical and radiologic improvements after treatment and were cured and who had a lower IFN-γ level, high C-reactive protein ≥ 3 mg/dl. These results also coincides with that of Pai et al. [36] Therefore the persistence of some symptoms and sign like (cough, dyspnea and rales) among non-responders may reflect continued inflammatory process and tissue destruction in the airways and lung parenchyma which may be attributed to uncontrolled infection and or required longer time of observation and anti TB treatment for reversion of their pathological symptoms and signs to normal state. In the present study the ESR first and second hour in active pulmonary tuberculosis groups (I&II) before starting treatment were significantly higher than ESR first and second hour after 3months of anti tuberculous treatment (groupII after treatment) which coincide with Lee et al [44] who demonstrated improvement in ESR after starting anti-TB treatment. Thus the elevation of ESR during tuberculous infection occurs as a result of the associated inflammation , however during the course of treatment it tends to decrease due to strong reduction of the inflammatory response under the effect of antituberculous treatment.[37] As regard sputum reversion for AFB half of group II (before treatment) patients to negative sputum for AFB after 3 months of first line of antituberculous treatment , however the other half did not show reversion, this may be explained by that these patients were MDR(multi resistant drug) patients or had dead bacilli in their sputum and /or the still having the infection and need more time for sputum conversion under the effect of antiTB treatment. There were no statistical significant differences between the studied groups regarding tuberculin test as positive tuberculin skin testing (TST) only indicates infection and by itself is not diagnostic of TB disease.[38] When tuberculin test was evaluated as a diagnostic test in relation to culture (table 4) it showed reduction in sensitivity 76.47% and specificity 66.67% in contrast to Lee *et al* [34] , who showed TST sensitivity for the diagnosis of active TB of 94% (95% CI, 87-98%), and specificity of 88% (95%CI, 74-96%) among young military personnel in South Korea. Our findings agrees with other studies [40-44] that showed that tuberculin test sensitivity was ranging from 70 to 78% albeit with a higher sensitivity rate ranging from 69%-88%. This could be explained by false-positive TST reactions due to BCG vaccination in infancy which become minimal later in young adults and the difficulties in TST which include the requirement for a return visit from the patient, variability in the application and evaluation of test results and differences in interpretation. IFN-γ release assays (IGRA) including the QuantiFERON-TB gold in-tube test (QFT-GIT) are increasingly used in place of the tuberculin skin test (TST) in surveillance programs for M. TB infection in the United States. However, data on conversions, reversions, and predictive value of QFT in such programs for health care workers (HCWs) are limited, [45] although there were no statistical significant differences between the studied groups regarding Quantiferon TB gold test. The Evaluation of QFT- Gold IT test as a diagnostic tool for TB in relation to culture in active TB groups (I, II) showed high sensitivity(91.18%) and high specificity(83.33%), similar to other studies [40,46,47] that reported QFT- Gold IT test sensitivity from 88% to 93% and specificity from 90% to 98%.The explanation of this higher observed sensitivity could be due to the characteristics of the participants, who were mostly young. As IGRA sensitivity has been reported to decrease significantly with older age [48], HIV infection [49], and chronic renal failure [50]. IGRA test in the present study showed higher sensitivity and specificity (table 4) when compared to Tuberculin skin test, which is similar to Diel *et al* and Hotta *et al.* [51,52] who concluded that IGRA test was a better indicator of LTBI than the TST test in BCG-vaccinated subjects. In addition to Ewer *et al* [53] concluded that The IGRA test was superior to the TST in terms of detecting contact with M. tuberculosis. Opposite to Adetifa *et al.,* [54] who found that IFN-γ-ELISPOT is probably not a useful biomarker of treatment efficacy in LTBI. Joshi *et al.,* [45] reported that Poor IGRA reproducibility and a low predictive value of QFT-GIT conversions indicate that QFT-GIT with current interpretation criteria should not be used for serial screening

of U.S. health care workers. Negative TST have higher reproducibility than QFT-GIT for serial testing of health care workers in low tuberculosis incidence settings. Poor agreement was found between Tuberculin test and QFT-Gold IT test where the Kappa (κ) was 0.025(table6) this is in contrast to Brock et al.,[55] who showed an excellent agreement between the TST and IGRAS tests (94%, κ value 0.866) The present study reported slow changes over time with no statistical difference in the IFN-γ levels of young active TB patients without any underlying disease, during treatment with anti-TB medication. These findings agree with previous studies [36,56] which showed a persistent IFN-γ response during treatment with positive QFT-G rates of 73% at baseline and 79% at 3 months, although the average IFN-γ level declined slightly. Also two other studies conducted in Japan [57,58] showed a progressive change of the IFN response after anti-TB treatment, with smaller percentages of subjects. The explanation of lower change rate after 3 months of anti-TB treatment in our study could be due to the higher initial IFN-g level (4.49 ± 3.85IU/ml) than those in Japan(0.51 ± 0.15 and 0.75 ± 0.15 IU/ml)[58],and may be attributed to the demographic characteristics of the participants, who were young, immunocompetent patients with no underlying co morbid disease. Another explanation may be shortage of the time of treatment so that the patients were incompletely cured, and should have to repeat the Quantiferon TB gold test after 6 months and12 months.

CONCLUSION AND RECOMMENDATIONS

IGRA test had high sensitivity and specificity in diagnosis of active TB and could be used with advantage in the diagnosis of LTBI in high-risk groups, either together with the TST and/or for the confirmation of the TST result. There is a need for studies of longer duration, observing the conversion of latent infection to active disease and using new diagnostic tests such as the IGRA test**.** Further studies are thus needed to evaluate changes in QFT-G IT results during therapy for active TB disease.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

REFERENCES

1. Philip A, Michael F, Kenneth G. Fishman's pulmonary diseases and disorders.New York: McGraw- Hill book Companies.2008; 2479 -85.
2. Lonnroth K, Raviglione M. Global Epidemiology of Tuberculosis: Prospects for Control. American Journal of Respiratory Critical Care Medicine 2008; 29:481–91.
3. World Health Organization. Global tuberculosis control 2009: Epidemiology, strategy, financing. World Health Organization. , Geneva, Switzerland 2011
4. Laskin D, Pendiono K. Macrophages and inflammatory mediators in tissue injury. Annals Revision of Pharmacology 1995; 135: 655-77.
5. Mosman T, Sad S. The expanding universe of T cell subsets: Th1, Th2 and more. Immunology Today 1996; 17: 138-46.
6. Trinchiri G. Cytokines acting on or secreted by maorophages during intracellular infection (IL-10, IL-12, INF- gamma). Current Opinion of Immunolology 1997; 9: 17-23.
7. Pai M, O'Brien R. New diagnostics for latent and active tuberculosis: State of the art and future prospects. Seminar in Respiratory Critical Care Medicine 2008; 29:560–8.
8. Ruiz J, Blanquer R, Lui J, Caminero J, Cayla J, Dominguez J, et al. Diagnosis and treatment of tuberculosis. Arch Bronconeumology 2008; 44(10):551-66.
9. Sahbazian B, Weis S. Treatment of active tuberculosis: Challenges and prospects. Clinics in Chest Medicine 2005; 26:273 –82..
10. Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB et al. Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. EurRespir J 2011; 37:100.
11. Pai M. Alternatives to the tuberculin skin test: Interferon γ assays in the diagnosis of Mycobacterium Tuberculosis Infection. Indian Journal of Medical Microbiology 2005; 23 (3):151-8.
12. Thillai M, PollockK , Pareek M, and Lalvani A. Reviews Interferon-gamma release assays for tuberculosis: current and future applications.Expert Review of Respiratory Medicine. 2014;8 : 67-78.
13. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O. et al.Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. ClinMicrobiol Rev. 2014;27:3.
14. Wallis RS, Pai M, Menzies D, Doherty TM, Walzl G, Perkins MD et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet 2010; 375:1920.
15. van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. PLoS One 2009; 4: 8517.
16. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. MMWR Recomm Rep 2010; 59:1.
17. Dheda K, van ZylSmit R, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. CurrOpinPulm Med 2009; 15:188.
18. Buttarello, M; Plebani, M.Automated blood cell counts: state of the art. American journal of clinical pathology 2008;130 : 104–16
19. Edward P. Liver function. In: clinical chemistry principles, procedures and correlations. Bishop ML, Edward PF, et al. 5th Ed. Lippincott Williams and Wilkins 2005; 475-95.
20. Lamb EJ and Price CP . Creatinine, urea and uric acid. In Tietz fundamentals of clinical chemistry. Burtis CA, Ashwood ER and Bruns DE. 6th Ed. Saunders Elseviers 2008;363-72.
21. Neeley WE. Simple automated determination of serum or plasma glucose by a hexokinase-glucose-6 -phosphate dehydrogenase method. ClinChem 1972; 18: 509-15.
22. Lee E, Holzman R. Evolution and current use of the tuberculin test. Clinics in Infectious Diseases 2002; 34:365-70.
23. Ronald W, Constance B. Preparation of acid fast microscopy smears for proficiency testing and quality control. Journal of Clinical Microbiology 1987; 6: 110-1.
24. World Health Organization. Global tuberculosis control 2009: Epidemiology, strategy, financing. World Health Organization. , Geneva, Switzerland 2011
25. Chee CBE, Gan SH, KhinMar KW, Barkham TM, Koh CK, Liang S and WangYT. Comparison of Sensitivities of Two

Commercial Gamma Interferon Release Assays for Pulmonary Tuberculosis.J. Clin. Microbiol. 2008 ; 46(6): 1935-40
26. Daly LE, Bourke GJ. Interpretation and uses of medical statistics. 5th ed. Oxford, Malden, MA: Blackwell Science; 2000.
27. Keeler E, Perkins MD, Small P, Hanson C, Reed S, Cunningham J, et al.Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature 2006;444:49–57.
28. Centers for Disease Control and Prevention. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm. Rep. 2005; 54:49–55.
29. Lange C, Pai M, Drobniewski F, Migliori GB. Interferon-gamma release assays for the diagnosis of active tuberculosis: sensible or silly? EurRespir J 2009; 33:1250–3.
30. Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB, et al. Interferon-{gamma} release assays for the diagnosis of active tuberculosis: a systematic review and metaanalysis. Eur Respir J. 2009; 33:1250–3.
31. Dheda K, van ZylSmit R, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. Curr Opin Pulm Med 2009; 15:188–200.
32. Denkinger CM, Dheda K, Pai M. Guidelines on interferon-c release assays for tuberculosis infection: concordance, discordance or confusion? Clin Microbiol Infect 2011; 6:806–14.
33. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: ametaanalysis. Chest 2010; 137:952–68.
34. Marciniuk DD, McNab BD, Tom Martin WMA, Hoeppner VH. Detection of Pulmonary Tuberculosis in Patients With a Normal Chest Radiograph. Chest. 1999;115(2):445-52.
35. Lee SW, Lee CT, Yim JJ. Serial interferon-gamma release assays during treatment of active tuberculosis in young adults. BMC Infectious Diseases 2010 ; 10:300.
36. Pai M, Joshi R, Bandyopadhyay M, Narang P, Dogra S, Taksande B et al. Sensitivity of a whole-blood interferon-gamma assay among patients with pulmonary tuberculosis and variations in T-cell responses during anti-tuberculosis treatment. Infection 2007; 35:98–103.
37. Suzuki K, Takashima Y, Yamada T, Akiyama J, Yagi K, Kawashima M et al. The sequential changes of serum acute phase reactants in response to antituberculous chemotherapy. Kekkaku 1992; 67(4): 303-11.
38. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion and reversion. Am J Respir Crit Care Med 1999; 159: 15-21.
39. Lee JY, Choi HJ, Park IN, Hong SB,Oh YM, Lim CM et al. Comparison of two commercial interferon-gamma assays for diagnosing Mycobacterium tuberculosis infection. Eur Respir J. 2006; 28 (1): 24 - 30.
40. Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K et al. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med. 2004; 170 (1): 59 - 64.
41. Kang YA , Lee HW , Yoon HI , Cho B, Han SK, Shim YS et al . Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA .2005 ; 293 (22): 2756 – 61.
42. Ferrara G, Losi M, D'Amico R, Roversi P, Piro R, Meacci M et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study .Lancet 2006 ; 367 (9519): 1328 - 34 .
43. Soysal A , Torun T , Efe S , Gencer H , Tahaoglu K , Bakir M Evaluation of cut-off values of interferon-gamma-based assays in the diagnosis of M. tuberculosis infection . Int J Tuberc Lung Dis .2008; 12 (1): 50 - 6.
44. Jafari C , Thijsen S , Sotgiu G , Goletti D, Domínguez Benítez JA, Losi M et al. Bronchoalveolar lavage enzyme-linked immunospot for a rapid diagnosis of tuberculosis: A TBNET Study. Am J RespirCrit Care Med .2009;180(7):666-673.
45. Joshi M, Monson TP, Joshi A, Woods GL. IFN-γ release assay conversions and reversions. Challenges with serial testing in U.S. health care workers. Ann Am Thorac Soc. 2014;11:296.
46. Markova R, Todorova Y, Drenska R , Elenkov I, Yankova M. Usefulness of interferon-gamma release asssys in the diagnosis of tuberculosis infection in HIV-infected patients in Bulgaria. Biotechnol Biotechnol Equip. 2009; 23 (1): 1103 - 8.
47. Aichelburg MC, Rieger A, Breitenecker F , Pfistershammer K, Tittes J . Detection and prediction of active tuberculosis disease by a whole blood interferon-gamma release assay in HIV-1-infected individuals .Clin Infect Dis .2009 ; 48 (7): 954 - 62 .
48. Kobashi Y, Mouri K, Yagi S, Obase Y, Miyashita N, Okimoto N, Matsushima T, Kageoka T, Oka M: Clinical utility of the QuantiFERON TB-2G test for elderly patients with active tuberculosis. Chest 2008, 133(5):1196-202.
49. Raby E, Moyo M, Devendra A, Banda J, De Haas P, Ayles H, Godfrey- Faussett P: The effects of HIV on the sensitivity of a whole blood IFN gamma release assay in Zambian adults with active tuberculosis. PLoS One 2008, 3(6):e2489.
50. Lee SS, Chou KJ, Su IJ, Chen YS, Fang HC, Huang TS, Tsai HC, Wann SR, Lin HH, Liu YC: High prevalence of latent tuberculosis infection in patients in end-stage renal disease on hemodialysis: Comparison of QuantiFERON-TBGOLD, ELISPOT, and tuberculin skin test. Infection 2009, 37(2):96-102.
51. Diel R, Ernst M, Doscher G, Visuri-Karbe L, Greinert U, Niemann S, et al: Avoiding the effect of BCG vaccination in detecting Mycobacterium tuberculosis infection with a blood test. EurRespir J 2006; 28: 16 – 23.
52. Hotta K, Ogura T, Nishii K, Kodani T, Onishi M, Shimizu Y et al. Whole Blood Interferon-Gamma Assay for Baseline Tuberculosis Screening among Japanese Healthcare Students. PLoS ONE 2007;2(8): e803.
53. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P et al: Comparison of T- cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak Lancet 2003; 361: 1168 –73.
54. Adetifa IM, Ota MO, Jeffries DJ, Lugos MD, Hammond AS, Battersby NJ etal. Interferon-γ ELISPOT as a biomarker of treatment efficacy in latent tuberculosis infection: a clinical trial. Am J Respir Crit Care Med. 2013 ;187:439-45
55. Brock I, Munk ME, Kok-Jensen A, Andersen P. Performance of whole blood IFN-gamma test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. Int J Tuberc Lung Dis; 2001; 5: 462–7.
56. Carrara S, , Vincenti D, Petrosillo N , Amicosante M, Girardi E, and Goletti D. Use of a T Cell–Based Assay for Monitoring Efficacy of Antituberculosis Therapy Clinical Infectious Diseases 2004; 38:754–6.
57. Kobashi Y, Obase Y, Fukuda M, Yoshida K, Miyashita N, Oka M: Clinical reevaluation of the QuantiFERON TB-2G test as a diagnostic method for differentiating active tuberculosis from non tuberculous mycobacteriosis. Clin Infect Dis 2006, 43(12):1540-46.
58. Kobashi Y, Sugiu T, Ohue Y, Mouri K, Obase Y, Miyashita N, Oka M: Longterm follow-up of the QuantiFERON TB-2G test for active tuberculosis disease. Intern Med 2008, 47(22):1957-61.