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Performance Of The Interferon Gamma Release Assays In Pulmonary Tuberculosis Patients In An Urban Metropolis

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Abstract
Interferon Gamma Release Assays (IGRAs) were developed for the indirect or immunologic diagnosis of tuberculosis infection; however, they have also been used to assist in difficult to diagnose cases of tuberculosis disease in adults, and to a lesser extent, in children, especially in those under 5 years old. Interferon-gamma release assay (IGRA)-positive rates and false-positive rates among the five age groups examined. Both rates were significantly different among age groups (all p-values <0.001). p-values were obtained by chi-square tests for trend. Interferon-gamma release assay (IGRA)-positive rates among the four anatomic types of uveitis and between patients with and without retinal vasculitis. The IGRA-positive rates were significantly different among the four anatomic groups (p = 0.001). Patients with retinal vasculitis had higher positive IGRA rate (p < 0.001) than those without retinal vasculitis. p-values were obtained using chi-square tests.

Keywords: Mycobacterium tuberculosis; drug resistance; blood; patients; IGRA

Introduction

India, with its populace of over 1000 million is probable to account for approximately 30% of the global tuberculosis burden (Dye et al., 1999). Tuberculosis (TB) remains to be major health problematic in India because of its high illness and humanity 9 (Murray and Lope, 1996). Around 2 billion, nearly one- third of the world inhabitants, are thought to be diseased with mycobacterium Tuberculosis In addition to the people with active TB, Many other are asymptomatic carriers (Latent TB) and many develop active TB at same tie in their lives (WHO, 2001). India account for closely one 3rd of the universal difficult of tuberculosis and it is a key barrier to socioeconomic growth along with being one of India's most important public health problem. In India tuberculosis kill 14 times more people than all tropic diseases combined, 21 times more than malaria and four hundred times more than leprosy. Every day in India more than 20000 people become infected

with tubercle bacillus, more than 5000 develop the diseases and more than 1000 die from tuberculosis. Every year another 20 lakhs people develop tuberculosis in India. The direct and indirect cost of TB to the country amount to Rs: 12000 crore (US dollar 3 billion) per year.

Tuberculosis (TB) is an infectious disease usually caused by the bacterium Mycobacterium tuberculosis (MTB). (WHO, October 2015) Tuberculosis generally affects the lungs, but can also affect other parts of the body. (WHO, October 2015) Most infections do not have symptoms, in which case it is known as latent tuberculosis. (WHO, October 2015) About 10% of latent infections progress to active disease which, if left untreated, kills about half of those infected. (WHO, October 2015) The classic symptoms of active TB are a chronic cough with blood-containing sputum, fever, night sweats, and weight loss. (WHO, October 2015) The historical term "ingestion" came about due to the weight loss. Infection of other organs can cause a wide range of symptoms. (Dolin et al, 2010). Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak, or sneeze. (CDC, March 2012). People with latent TB do not spread the disease. (WHO, October 2015) Active infection occurs more often in people with HIV/AIDS and in those who smoke. (WHO, October 2015). Diagnosis of active TB is based on chest X-rays, as well as microscopic examination and culture of body fluids(Konstantinos A,2010). Diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests. (Konstantinos A,2010). Prevention of TB involves screening those at high risk, early detection and treatment of cases, and vaccination with the bacillus Calmette-Guérin (BCG) vaccine. (Hawn TR et,al, 2014) (Harris and Randall E. 2013) (WHO, October 2008) Those at high risk include household, workplace, and social contacts of people with active TB. (WHO, October 2008) Treatment requires the use of multiple antibiotics over a long period of time. (WHO, October 2015) Antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). (WHO, October 2015). Presently, one-third of the world's population is thought to be infected with TB. (WHO, October 2015) New infections occur in about 1% of the population each year. In 2016, there were more than 10 million cases of active TB which resulted in 1.3 million deaths. This makes it the number one cause of death from an infectious disease. More than 95% of deaths occurred in developing countries, and more than 50% in India, China, Indonesia, Pakistan, and the Philippines. The number of new cases each year has decreased since 2000. (WHO, October 2015) About 80% of people in many Asian and African countries test positive while 5–10% of people in the United States population test positive by the tuberculin test.(Kumar V et, al,2007). Tuberculosis has been present in humans since ancient times (Lawn SD and Zumla AI, July 2011).

Timely and accurate diagnosis of tuberculosis (TB) disease in patients must be given a high priority by medical practitioners for the following reasons: global burden of TB disease children carry 6% of the global burden of TB disease; more likely to develop the most severe forms of disseminated and meningeal TB, which is due to the immature immune system.However, diagnosis of TB is challenging because symptoms are often non-specific, specimens may be difficult to obtain, and

bacteriological confirmation is less frequent than in adults. Furthermore, children younger than 5 are more likely to have severe extra-pulmonary TB while the most severe cases of TB are often seen in infants. For these reasons, diagnosing TB in patients warrants additional efforts.Interferon gamma release assays (IGRAs) are promising alternatives to the tuberculin skin test (TST). However, few studies have investigated their use in young children and infants. Consequently, guidelines from the American Academy of Pediatrics state that IGRAs are not recommended for routine use in children younger than five years of age due to a lack of published data.IGRAs have been used mainly in the indirect or immunologic diagnosis of tuberculosis infection. They also can be used to assist in a diagnosis of tuberculosis disease in cases that are difficult to obtain a microbiological diagnosis or that need early diagnosis. Therefore, we have performed a study in a hospital setting to help provide the accuracy of an IGRA in the tuberculosis patients in an urban metropolis

Methods

Study population and methods: The 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipatti patients (hospitalized in Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions. All 934 cases were subjected to a drug-resistance test. The anti-microbial drug susceptibility tests (DST) were performed using the WHO standard conventional proportional method.

Specimen collection, storage, and handling procedures; criteria for specimen rejection: Collect 1 ml of blood by venipuncture directly into each of the QuantiFERON TB Gold IT blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube. Tubes should be between 22° C + 5° C at the time of blood draw. Immediately after filling tubes, shake them ten times just firmly enough to ensure the entire inner surface of the tube is coated with blood, to solubilize antigens of tube walls. Over energetic shaking may cause gel disruption and could lead to aberrant results. Ship tubes to laboratory at 22° C + 5° C as soon as possible and within 16hrs of collection. Do not refrigerate or freeze the blood samples. The assay is set up at least once a week or more frequently depending on workload. Samples are stored for a minimum 7 days at 2° - 8° C after result have been posted.

Blood cultures:Blood cultures using mycobacteria-specific, radioisotope-labeled systems help to establish the diagnosis of active TB. However, mycobacterial bacteremia (bacillemia) is detectable using blood cultures only if specialized systems are used; these bacilli have specific nutrient growth requirements not met by routine culture systems. Such blood cultures should be used for all patients with HIV infection who are suspected of having TB, because bacillemia is particularly prevalent in this population. If available, in fact, these cultures should be used for any patient highly suspected of having active TB.

Statistical analysis

Data obtained from medical records were entered and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). The sensitivity of each IGRA among the different age groups was compared using binary logistic regression and linear-by-linear association. Comparisons of continuous variables including WBC and lymphocyte counts, CRP, serum protein, and serum albumin levels, across age groups were performed using one-way analysis of variance (ANOVA) and post-hoc analysis. The effect of each factor on the sensitivity of each IGRA was analyzed by logistic regression adjusting for age group. A factor was considered to influence IGRA sensitivity when the age group was adjusted by a certain variable or some variables and the sensitivity of the IGRA according to age group was statistically insignificant. A p value less than 0.05 was considered significant.

Results

During the research period, 934 pulmonary TB patients were studied, of which 274 (29.33 ± 1.5%) ($p < 0.001$) men in the age group 25–65years outnumbered women between 2.7 and 9.0 times more; 660 (70.66 ± 1.5%) of the TB cases were men. In the age group <15–24, as well as in the age group over 65 years, the proportion of men and women were similar. In the remaining age groups, the proportion of men with TB was significantly higher than women. The total ratio of male TB patients among the female patients of all groups surveyed in 2007 was 2.4, which agrees with the WHO European Region. In the age group 45–54 the male to female ratio was the highest among patients with TMDR-TB.Multiple logistic regressions were performed to identify factors associated with the IGRA results (Table 1 and Fig. 1). Age was independently associated with a positive IGRA result ($p < 0.001$), but the anatomic type of inflammation was not ($p = 0.176$). The odds ratio for age adjusted for presumed TRU and anatomic type was 1.06 (95% confidence interval [CI], 1.03 to 1.09), and that for age group adjusted for the same confounding variables was 1.90 (95% CI, 1.41 to 2.56; $p < 0.001$). Likelihood ratios were calculated for assessing the value of performing the IGRA diagnostic test. The positive likelihood ratio was 3.57 and the negative likelihood ratio was 0.00 in our study.

Table 1: Interferon gamma release assay

	All patients	IGRA result			Presumed TRU		
		Positive	Negative	p-value	TRU	Non-TRU	p-value
	(n = 181)	(n = 65)	(n = 116)		(n = 20)	(n = 161)	

Age (yr)	43.4 ± 16.2	50.0 ± 13.5		39.7 ± 16.4		<0.001*	45.6 ± 14.6		43.1 ± 16.4	0.531*
Sex (male : female)	99 : 82	37 : 28		62	: 54	0.652†	10 : 10		89 : 72	0.655†
Positive IGRA result (%)	65 (35.9)	65	(100)	0		NA†	20	(100)	45 (28.0)	<0.001†
Anatomic type (%)										
Anterior	75 (41.4)	20	(30.8)	55	(47.4)		3	(15)	72 (44.7)	
Intermediate	34 (18.8)	9	(13.8)	25	(21.6)	0.001†	0		34 (21.1)	<0.001†
Posterior	49 (27.1)	29	(44.6)	20	(17.2)		15	(75)	34 (21.1)	
Panuveitis	23 (12.7)	7	(10.8)	16	(13.8)		2	(10)	21 (13.0)	
With retinal vasculitis	32 (17.7)	23	(35.4)	9	(7.8)	<0.001†	12	(60)	20 (12.4)	<0.001†

IGRA = interferon gamma release assay; TRU=tuberculosis uveitis; NA=not applicable

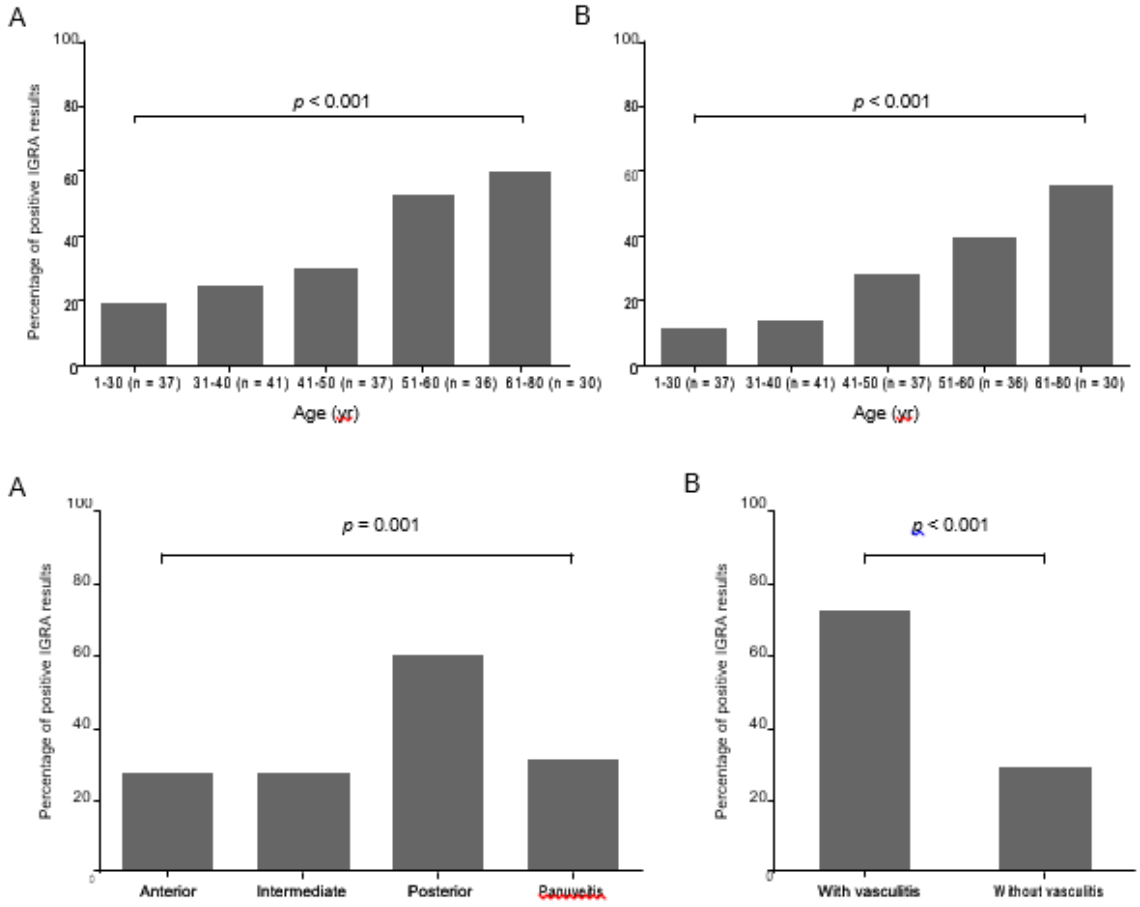

Fig. 1. Interferon-gamma release assay (IGRA)-positive rates (A) and false-positive rates (B) among the five age groups examined. Both rates were significantly different among age groups (all p-values <0.001). p-values were obtained by chi-square tests for trend. Interferon-gamma release assay (IGRA)-positive rates among the four anatomic types of uveitis and between patients with and without retinal vasculitis. (A) The IGRA-positive rates were significantly different among the four anatomic groups (p = 0.001). (B) Patients with retinal vasculitis had higher positive IGRA rate (p < 0.001) than those without retinal vasculitis. p-values were obtained using chi-square tests.

Discussion

The accuracy and reliability of IGRAs among the pulmonary cases in hospital settings is not yet well defined. The study selected 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipatti patients (hospitalized in `Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions. Intraocular inflammation can have many different origins and presents with a wide spectrum of clinical manifestations. If the

underlying inflammatory cause is treat-able, prompt and appropriate treatment can result in a favorable outcome. In the current study, we examined the IGRA as a diagnostic tool for presumed TRU in Korean patients with intraocular inflammation. Our results suggest that the IGRA is highly sensitive and moderately specific for TRU (Groenen et al.,1993). A test used for TB screening should have good sensitivity and acceptable specificity, so we believe that IGRA can be used as a screening test for presumed TRU in Korea. Unfortunately, the TST (Mantoux test) is not useful as a screening tool because of the high false-positive rate in the Korean population, which has a very high BCG vaccination rate. Therefore, an alternative test is needed for the Korean population, and in this study, we evaluated the IGRA as a screening method for TRU. However, the false-positive rate (1-specificity) of IGRA was not low and cannot be neglected. Therefore, clinicians should consider which patients are more likely to have true TRU. Our analyses showed that younger age ($\leq$ 40 years), a positive IGRA, and the presence of posterior uveitis and retinal vasculitis were all predictive of TRU (Kenyon et al., 1997; Feng et al., 2009).

Broad-based posterior synechiae, retinal vasculitis (with or without choroiditis), and serpiginous choroiditis in patients with latent or manifest TB are clinically suggestive of TRU in TB-endemic areas (Kang, 2005). This may explain why the positive predictive value of IGRA was greater in patients with posterior uveitis and retinal vasculitis. Sever-al studies have reported that vasculitis can occur from a TB infection, and it may be associated with hypersensitivity to MTB (Godfrey-Faussett, P. and Stoker, N.G.1992). We examined how a positive IGRA result should be interpreted in patients with suspected TB uveitis. Because our older patients tended to have a higher IGRA positivity rate, a positive result is likely insufficient to make a definitive TRU diagnosis, especially in elderly patients with intraocular inflammation. This result is comparable to those of (Kang et al.1980), who reported that positive IGRA rates are positively and linearly correlated with patient age. In our study, the age of patients with and without TRU were not significantly different, so this relationship did not result from inherent group differences in age (Hensel et al., 2016). We also examined which patients should have the IGRA performed for diagnosing presumed TRU. Patients with intraocular inflammation usually undergo extensive diagnostic workups to identify the underlying inflammatory cause (Poulet et al., 1995). Performing the IGRA on all patients with intraocular inflammation would not be cost-effective. Therefore, we recommend having the IGRA done for patients with a clinical presentation suggestive of TRU, including retinal vasculitis and posterior uveitis. In addition, because a positive IGRA result in a young patient with posterior uveitis or retinal vasculitis likely indicates TRU, the test should be heavily considered if a concurrent TB infection is suspected (Rajendran et al., 2011;Rodrigues et al., 1990).

Conclusion

Tuberculosis remains one of the most-deadly infectious diseases and has claimed millions of lives for manyyears. While significant progress has been made towardscontrolling the global burden of TB over the past decade,more efforts are still needed. Emerging issues such as multidrug-

resistance threatens to revert the progress maderegarding TB care and control. The knowledge base for TB remains a rapidly expanding area and global guidelines arecontinually being refined for instance to incorporate newanti-tubercular drugs to tackle issues of resistance. Health professionals, policy makers, patients and the general public need to keep up-to-date with current trends in TBmanagement and control. This will be essential for efficient adoption of global guidelines to country-level situation, particularly taking into consideration issues such as diseaseburden, health system structures and available resources.

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