DOI: 10.5958/2319-5886.2015.00067.3

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 4 Issue 2 Coden: IJMRHS Copyright @2015 ISSN: 2319-5886

Received: 14th Jan 2014 Revised: 10th Jan 2015 Accepted: 27th Feb 2015

Research article

COMPARATIVE STUDY FOR EVALUATING T SPOT-TB IN ANALYSIS BETWEEN LOW AND HIGH RISK SUBJECTS - A PILOT STUDY

*Gupta V[1], Athavale A.U[2], Natraj G[3]

[1]Registrar, [2]Professor and Head, Department of Pulmonary Medicine, [3]Professor, Department of Microbiology, Seth Gordhandas Sunderdas Medical College and King Edward Memorial Hospital, Mumbai

*Corresponding author email: drvishalgupta1985@gmail.com

ABSTRACT

Latent tuberculosis infection (LTBI) is a non- communicable asymptomatic condition, which might develop into active tuberculosis. Health care workers in contact with active TB cases are at high risk. Impact of exposure in high TB endemic population remains to be studied. **Objective:** To study the prevalence of IGRA positivity in high risk health care worker and comparing with clinical and radiological data. **Methods**: From a tertiary care institute, 40 subjects of which low risk subjects (16), high risk subjects / health care workers (24) were recruited randomly. TSPOT TB spot counting was done and correlation with the clinical data and radiology was analysed. **Results:** Out of 18 positive results, 16 were HCWs (66.67%), 2 were of low risk group (12.5%). Among the HCWs, doctors had the maximum percentage of the positive results (71.42%). Administration related workers all had negative results. Correlation was established between different antigens used. 8 subjects with normal chest x ray also had TSPOT positive result. **Conclusion:** HCW's especially those proximally exposed are at greater risk of having positive T SPOT assay. Chest X ray may not be an adequate screening tool. The exact significance and clinical implication need study even in high endemic population.

Keywords: IGRAS, Interferon-γ, Health Care workers, Latent Tuberculosis

INTRODUCTION

Latent tuberculosis infection (LTBI) is a non-communicable asymptomatic condition, which can develop into active tuberculosis months or years later [1]. There are two principle approaches for tests used in clinical practice to detect latent infection with M. tuberculosis. These are, the in vivo tuberculin skin test (TST), which uses a mixture of antigens obtained as a protein precipitate from the liquid cultures of M. tuberculosis, and the *ex vivo* interferon-γ release assays (IGRAs), which are designed to identify a memory of an adaptive immune response against mycobacterial antigens [2].IGRAS most popularly available includes the T SPOT TB™ and the Quantiferon Gold (QFT-G™). T SPOT-TB™ measures release of IFN-γ from sensitized lymphocytes in vitro. Although TST and IFN-γ assay use different antigen combinations, these tests had comparable prevalence estimates (41% and 40%, respectively) and a high level of agreement [3]. T-SPOT. TB is intended for use for detection of *M.* tuberculosis infection in conjunction with risk assessment, radiography and other medical and diagnostic evaluation [4]. The use of the Tuberculin skin test in diagnosing active infection is limited to the category of people who have high levels of exposure to tubercular antigens like the health care worker [5,6].

A community based tuberculosis elimination strategy will require a reduction in the prevalence of infection with M. tuberculosis through identification of latent

TB infection (in future possibly treatment) that may later develop into active tuberculosis disease [7], thus possible early diagnosis of active disease. An Indian study by Mahomed et al. showed that there was poor agreement between TST and QFT tests, and also between the different generations of QFT tests (K = 0.12-0.50). Of the subset with TST indurations >15 mm, 30- 56% had negative QFT test [8]. A study conducted by Pai et al.[9] on health workers in India demonstrated a high prevalence of LTBI in Indian health care workers as has been in studies from around the world[10]. Increasing age and years in the health profession were risk factors for both IFN-γ assay and TST positivity, and the risk factor associations were fairly similar for both tests [3,10].

Aims and Objectives: To determine the clinical utility and prevalence of significant result of T-SPOT.TB™ in health care workers. To determine the extent of correlation of A and B antigen wells of TSPOT test.

MATERIAL AND METHODS

Research Design: Prospective observational study

Sample size: A total of forty subjects were included of which 16 were categorized as low risk subjects (group 1) and 24 as high risk subjects / health care workers (group 2).

Sampling Method: Purposive sampling; non random-mised.

Inclusion criteria: Health Care workers between the ages of 18-60 years and consenting for the study. In order to rule out disease in the subjects, history, clinical examination, chest radiography, blood counts and sputum evaluation was done.

Exclusion criteria: History of past or current treatment with anti-tuberculosis drugs or any other active disease state. Low risk subject: Subject not involved in active patient care, thus unlikely to be having exposure to tubercle bacillus more than baseline population.

High risk subject: Subject involved in the active patient care having high likelihood of contact with tubercle bacillus.

Study period: 2 years from obtaining the ethical committee approval. (June 2012).

Procedure: The subjects (male and female) working in all departments in a tertiary care hospital were recruited for the study after consenting as per the institutional ethical committee requirements. They were grouped into high and low risk categories (CDC 2010 criteria) [4] based on their risk of exposure to sputum AFB positive cases in the hospital. After detailed history and examination, the blood sample was drawn and the T SPOT TB™ test was done on the heparinised sample within an average time span of 6 hours. The T SPOT TB™ test was performed as per the kit literature provided with PANEL A representing the ESAT -6 and PANEL B representing the CFP-10 (TB specific antigens). (as per kit literature for TSPOT TB™)

The results of the TSPOT TB™ of the serum sample obtained from both groups one and two were tallied and analysed using chi square test of analysis on the SPSS data analysis software. $p<0.05$ was considered significant.

RESULTS

Of the total 40 subjects tested (Table1), a positive T SPOT TB result was detected in 16 subjects (66.67%) of the high risk group and 2 (12.5 %) in the low risk group.

Table1: Risk v/s Result of T SPOT TB™

RISK	RESULT OF T SPOT TB ™		Total
	Positive	Negative	
High	16 (66.6%)	8(33.33%)	24
Low	2(12.5%)	14(87.5 %)	16
Total	18	22	40

The difference was found to be statistically significant. (p=0.002) (Table2)

Table 2: Chi-square test for the statistical analysis of the study

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	9.82[a]	1	.002		
Continuity Correction	7.88	1	.005		
Likelihood Ratio	10.73	1	.001		
Fisher's Exact Test				.003	.002
Linear-by-Linear Association	9.57	1	.002		
No. of Valid Cases	40				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.80.
b. Computed only for a 2x2 table

Comparing the cadre of work with the results of the T SPOTB ™ test (Table 3), it was observed that doctors had the maximum percentage of positive results (71.42%) followed by the labour class workers (61.53%) whereas the administrative workers who had minimal exposure to active TB cases, had no positive results. The labour class workers who were directly exposed to the patients in ward activities were found to be all positive for TSPOT TB™.

Table 3: Comparing the result and the cadre of work

cadre of work	Result of T SPOT TB		Total
	Positive	Negative	
Doctor	5 (71.42%)	2 (28.6%)	7
Staff nurse	5 (50%)	5 (50%)	10
Class Four Worker Ward work Administrative work	 8 (61.53%) 0 (0%)	 0 (0 %) 5 (38.47%)	13
Administration	0 (0)%	10 (100%)	10
Total	18	22	40

Comparing the findings of Chest X ray abnormalities and the TSPOT TB (Table4), the one subject with the upper zone opacities had a strongly positive TSPOT TB™. There were 8 subjects who had no symptoms and had normal chest x-ray, but still had positive T SPOT TB results.

Table 4: CXR findings v/s Result of T SPOT TB™

CXR findings	Result of T SPOT TB		
	Positive	Negative	Total
CXR not available	9	7	16
Minimal UZ infiltrates	1	0	1
Normal	8	14	22
Opacities	0	1	1
Total	18	22	40

Comparing the results of the individual antigen panels A and B (TABLE 5), it was observed that there was good correlation between the two panel antigen tests as a majority (11 subjects) had both titres positive (>10 spots).

Table 5: Panel A and B assays as compared with the results of the test

PANEL A spots	PANEL B spots		
	< 6	6-10	>10
< 6	22	0	2
6 to 10	0	4	1
>10	0	0	11

NEGATIVE RESULT
POSITIVE RESULT

However, one subject had low PANEL B titre while 2 subjects had low PANEL A titres. Thus, a low cut off value at 6 is likely to optimise the sensitivity while maintaining the specificity of the test. Likely a lower cut off value will be helpful in a high incidence setting. Correlation between the titres and the magnitude of the exposure will need further study.

Further evaluation of the utility of the test to detect the latent TB cases in the high risk population especially doctors, staff nurses and the labour class workers will be needed

DISCUSSION

T SPOT-TB™ measures release of IFN-γ from sensitized lymphocytes in vitro. These tests have enhanced specificity because they selectively detect responses to CFP-10 and ESAT- 6, antigens secreted by M. tuberculosis that are not present in BCG, hence diminishing false positives seen in TST due to prior BCG vaccination and NTM[11]. Therefore, in countries with adequate resources, QFT-G™ or T SPOT-TB™ may ultimately replace skin testing in the diagnosis of latent TB infection while also serving as an adjunct in the diagnosis of active TB by conducting risk assessment, radiography and other medical and diagnostic evaluations [4]. In light of the above, it was worthwhile considering the specific tests like IGRAS in the evaluation of high risk candidates (health care workers) and comparing its titres with the low risk population.

For IGRAs, the reported sensitivity of T-SPOT was highest, reaching a pooled value of 87.5% while pooled sensitivity from the QFT-IT studies was 81%. This figure is remarkably different from the pooled sensitivity of 70% based on the six QFT-IT studies referenced by Pai et al [12]. Because most of the T-SPOT studies with respect to sensitivity were also performed in developed countries, the pooled sensitivity estimate for QFT-IT increases to 84.5% (and 88.5% for T-SPOT) in developing countries [13]. In the current study, it was shown that the probability of the subject having the low risk and the negative result (specificity) was about 87.5% which was close to the figure projected by the study by Pai et al.[9] The sensitivity (positive TSPOT TB test in high risk candidates) is likely to be low, in this case is about 66.6 % from the total population considered. The significantly lower sensitivity of the IGRAS observed in this study and few other studies from resource

limited settings needs further evaluation and should be addressed in upcoming studies with larger sample size [14]. Studies with patients have attributed this to the immunologic status of patients in such settings i.e., HIV co infection, advanced disease, or malnutrition) and to logistic requirements of the studies [14]. QFT-IT cut off is drawn to achieve maximum specificity, whereas the commonly used European T-Spot cut off of 6 spots appears to maximize sensitivity [13]. No such cut off values have been set for high burden, high endemic countries. Thus, the current study has also used the cutoff of TSPOT-TB™ test as per the European standards. The results of the same have been satisfactory but need confirmation in larger trials.

CDC USA guidelines [4] suggest that QFT-G can be used in place of the TST for infection control surveillance, and conversion (i.e. new infection) has been defined as change from a negative to a positive result. The UK National Institute for Health and Clinical Excellence (NICE) TB guidelines were published in March 2006 [15]. This guideline recommends a two-step (hybrid) strategy for LTBI diagnosis: initial screen with TST and those who are positive (or in whom TST may be unreliable) should then be considered for IGRA testing, if available, to confirm positive TST results. There are no consensus guidelines in India for the IGRAS but for serial testing of health care workers, the IFN-γ assay will be appropriate [16]. It will eliminate the need for repeat visits, avoid boosting, and minimize interpretational difficulties. [7,17]. However; the limited evidence on the use of IGRAs in serial testing of healthcare workers suggests that the diagnostic threshold for conversion does not take into account the possibility of misclassifying nonspecific IFN-γ changes as true conversions [3,18].

Several studies have shown that working in healthcare is a well-known risk factor for TB infection [9, 10, 19, 20, 16, 5, 6]. However, older studies did not test for LTBI [3, 18]. Positive result from the current study 66.67 % of high risk subjects being positive against the 12.5 % of low risk subjects. TST and the IGRAS conversion may have significance in detecting active cases but IGRAS yield fewer false positives in the BCG vaccinated HCWs.[12,20]

In the current study, the subject with upper zone opacity on chest X ray was found to have a positive T SPOT test while those with normal chest X ray had a significantly positive result in 8 of 22 subjects (36.36%). This is likely to represent the load of latent TB infection in this population. The prevalence of the TSPOT TB positive result in our study is 45 % (TABLE1). Which correlates with the study by Pai et al. [9] might be an underestimate because of the varied people involved in the health care and the differing degree of exposure to TB bacilli. Although age and years in health care reflect cumulative exposure to M. tuberculosis, variability of risk across job categories as both these studies may reflect variations in exposure frequency and intensity. Lalvani et.al [21] showed the prevalence of LTBI to be 80% in healthy adults (affluent corporate executives) in Bombay who were Enzyme-Linked Immuno Spot positive to either ESAT-6 or CFP-10. In contrast, only 55.8% of our high-risk cohort was positive by IFN-γ assay. The epidemiological estimate for LTBI in India is 41% TST positivity [21].

The limitations to the TPSOT TB testing in general have been:

Cost of testing is extremely high.

Skilled lab technicians and laboratory setup are needed. Not all the laboratory reagents and reading equipment are included by the manufacturer. Inter observer variability is high.

There is no true way of differentiating the latent and the active TB infection i.e. lack of a gold standard.

TST has higher sensitivity according to many studies as compared to the IGRAS. But some newer trials have suggested that the true positives may in fact be higher for the QFTG-IT and the TSPOT TB tests for variable reasons. [3, 19] Thus, while TST conversion remains as an indication for treatment, the same cannot be reliably stated for TSPOT TB. Association between the conversion and benefit of therapy still remain to be proven. Thus, more studies are needed to evaluate the transmission of the TB bacillus in the nosocomial setting.

Cut off titres for TSPOT TB and definition of conversion in high TB prevalent population are yet to be confirmed.

Guidelines from low income and high prevalence countries are needed for the screening and initiation of treatment of LTBI. The value of these tests in follow up of patients has an added value in TB management. Cost-effectiveness of the IGRAS in various settings (low and high risk populations) will need to be studied.

Overall, our findings highlight the need to study tuberculosis among Indian health care workers. The safety and well-being of health care workers is important for the continued expansion of the TB program.

The current study consisted of health care workers in a high prevalence country. Although this may limit our ability to generalize the results to settings with different baseline prevalence, we believe our data will be helpful in understanding the performance of IGRAS in high-burden settings for which data are scarce. The results and the available evidence suggest that the test has its advantages and limitations and, as of this time, it may have a useful role, depending on factors unique to each setting.[3] Subjects from the high risk group who had a positive result later developed constitutional features and radiological manifestation of disease activity and responded to ATT.

CONCLUSION

The study highlights the high prevalence of latent TB infection especially in high risk groups such as health care workers (66.5 % positive in the health care workers as compared to 12.5 % in low risk population). Since the low risk group had most negatives, the test is likely to have a high specificity. Even though there is sparse data to allow broad application, the correlation between positive and negative results is significant and should prompt physicians to evaluate such individuals to be evaluated for active / latent disease with sputum and radiography.

ACKNOWLEDGEMENT

I am extremely grateful to the staff, fellow residents and administration for their valuable support in the study. I am also grateful to the ICMR (Indian Council Of Medical Research) and the Diamond Jubilee Research Trust, KEM Hospital and RNTCP (DOTS) programme for the support with funding of this project. Special mention to Dr. Rupali Suryavanshi (Asst. Professor, Microbiology) and Dr. Jairaj Nair (Asst. Professor, Pulmonary Medicine) for their valuable help and technical expertise from time to time.

Conflict of Interest: Nil

REFERENCES

1. World Health Organization. Guidelines for the Prevention of Tuberculosis in Health Care Facilities in Resource-Limited Settings. Geneva, Switzerland: World Health Organization; 1999; 269: 8-13.
2. Lalvani A, Millington KA. T cell-based diagnosis of childhood tuberculosis infection. Curr Opin Infect Dis 2007; 20: 264–71.
3. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part I. Latent tuberculosis. Expert Rev Mol Diagn. 2006 ; 6 (3):413-22.
4. Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection - United States, 2010. CDC Gerald H. Mazurek, John Jereb, Andrew Vernon,et al. June 25, 2010;59;1-25. 21.
5. Christopher D.J, Daley P, Armstrong L , James P, Gupta R, Premkumar B,et al. Tuberculosis Infection among Young Nursing Trainees in South India. PLoS One. 2010; 5(4): 10408.
6. Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. PLoS Med. 2006;3: 494.
7. Rieder HL. Reconciling historical epidemio-logical, bacteriological and immunological observations in tuberculosis. Int J Epidemiol 2008; 37: 932–34.
8. H. Mahomed et al. Comparison of Mantoux skin test with three generations of a whole blood IFN-γ assay for tuberculosis infection. Int J Tuberc Lung Dis 2006; 10(3): 310-16.
9. Pai M, Gokhale K, Joshi R et al. Mycobacterium tuberculosis infection in health care workers in rural dia: comparison of a whole-blood, interferon-γ assay with tuberculin skin testing. JAMA 2005 (293), 2746–2755.
10. J. Torres Costa, R. Sa, M.J. Cardoso, et al Tuberculosis screening in Portuguese healthcare workers using the tuberculin skin test and the interferon-γ release assay, Eur Respir J 2009; 34: 1423–28.
11. Juan Carlos Palomino, Sylvia Cardoso Leão, Viviana Ritacco “From basic science to patient care.” Tuberculosis 2007.184-85.
12. Madhukar Pai, Guidelines on IGRAS cordant or discordant 2nd symposium on IGRAS. 2009.

13. R. Diel, R. Loddenkemper, and A. Nienhaus, Evidence-Based Comparison of Commercial Interferon-γ Release Assays for Detecting Active TB- A Metaanalysis chest 2010 ;137 (4): 952-68.
14. Dick Menzies, Madhukar Pai, and George Comstock Meta-analysis: New Tests for the Diagnosis of Latent Tuberculosis Infection: Areas of Uncertainty and Recommendations for Research. Ann Intern Med. 2007; 146: 340-54.
15. National Institute for Health and Clinical Excellence. Tuberculosis: Clinical Diagnosis and Management of Tuberculosis, and Measures for its Prevention and Control. London, National Institute for Health and Clinical Excellence, 2006. www.nice.org.uk.
16. Pai M, Joshi R, Dogra S, Mendiratta DK, Narang P, Kalantri SP, Reingold AL, Colford JM, Riley LW, Menzies D: Serial testing of health care workers for tuberculosis using interferon-g assay. American Journal of Respiratory and Critical Care Medicine 2006 (174): 349-55.
17. Gopinath KG, Siddique S, Kirubakaran H, Shanmugam A,Mathai E, Chandy GM. Tuberculosis among healthcare workers in a tertiary-care hospital in South India. J Hosp Infect. 2004; 57:339-42.
18. Jasmer RM, Nahid P, Hopewell PC. Clinical practice. Latent tuberculosis infection. N Engl J Med 2002; 347(23):1860–6.
19. Rao KG, Aggarwal AN, Behera D. Tuberculosis among physicians in training. International Journal of Tuberculous Lung Disease 2004; 8:1392-94.
20. Madhukar Pai, JessicaMinion, Hojoon Sohn, et al Novel and Improved Technologies for Tuberculosis Diagnosis: Progress and Challenges, Clin Chest Med 2009; 30: 701–16.
21. Lalvani A, Nagvenkar P, Udwadia Z. et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. J Infect Dis. 2001; 183: 469-77