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"Efficacy of IGRA in the Diagnosis of Tuberculosis and its Correlation with Fluorescence Microscopy and Chest X-Ray in a Tertiary Care Setting in Sikkim"

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Abstract

Background: *Studies have shown Interferon gamma release assay as an aid in diagnosing Mycobacterium tuberculosis infection, including latent tuberculosis infection. This study intended to evaluate the efficacy of IGRA in case detection of pulmonary tuberculosis and correlation with fluorescence microscopy and chest x-ray findings.*

Methods: *Clinically suspected patients (N=300) of pulmonary tuberculosis with chest x-ray findings were screened by fluorescence microscopy and IGRA. Each patient was subjected to IGRA, to diagnose active as well as latent infection.*

Results: *Overall 300 patients were enrolled. A total of 89% correlation was observed between IGRA and fluorescence microscopy with the two-sided P-value=0.0022, considered very significant. 100% correlation was observed between chest x-ray results and IGRA. However, 63% correlation was observed between fluorescence microscopy and Chest x-ray findings. IGRA was found to be 100% sensitive and 99.09% specific and an efficacy of 100%. However, fluorescence microscopy showed a sensitivity of 62.5%, specificity of 100%, with an efficacy of 90%.*

Conclusion: *IGRA is a reliable tool for diagnosis as compared to fluorescence microscopy. It is more sensitive, specific and accurate than fluorescence microscopy. It can be a better diagnostic tool in case detection among children.*

Keywords: *IGRA, Fluorescence microscopy, Chest x-ray, TB.*

Introduction:

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (MTB), affecting the lungs,[1] producing either a silent latent infection or a progressive active disease. In a healthy person, infection with MTB often causes no symptoms, since the person's immune system acts to "wall off" the bacteria.[2] Unlike the risk of acquiring infection with MTB, the risk of developing disease after being infected depends largely on, which includes, the number of MTB organisms inhaled (infecting dose), the virulence of these organisms and the host's cell-mediated immune response.[2] Clinical illness directly following infection is classified as primary TB and is common among children up to 4 years of age, which is severe and disseminated and it is usually not transmissible. When infection is acquired late in life, the immune system will contain it, at least temporarily. Dormant bacilli may persist many years before reactivation to produce secondary (post primary) TB, which is often infectious. Overall, it is estimated that ~10% of the infected youth population will eventually develop active TB.[3]

Recently, IGRA has shown its superior diagnostic performance over Tuberculin skin test by using two specific antigens (ESAT-6 and CFP-10) present exclusively in MTB but absent in BCG strains and most non tuberculosis mycobacteria.[4] IGRA does not measure and provide information about the presence or absence of the infecting organism rather measures the host cell immune response.[5] However, the sensitivity of IGRAs available commercially for diagnosing active TB was reported to be 85-93%.[6-10],[17] QuantiFERON technology (QFT) is a unique approach, based on IGRA. IGRAs are blood tests that measures the cell-mediated immune response of TB in infected individuals. Approved by US FDA, EU (MDSS), Australia, Japan notifying that QFT is an indirect test for MTB infection and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations. It is a whole blood test that can aid in the

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diagnosis of MTB infection, including both latent tuberculosis infection (LTBI) and TB disease. IGRA measures a person's immune reactivity to MTB.[7] T-lymphocytes in most person's that have been infected with MTB will release interferon gamma (IFN-γ) when mixed with antigens derived from MTB.[8] The testing culture tubes of the Immune Check TB Platinum Kit contains two gene recombinant antigens of the early secretory antigenic target 6kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10) encoded I region difference 1 (RD1), which are specific to pathogenic MTB but lack in BCG and other mycobacteria, then the test is followed to quantitate the amount of IFN-γ released.[9],[10] IGRA is known to be accurate in diagnosing LTBI but the efficacy of IGRA in diagnosing an active infection still remains an unsolved question. India is accounted for being one of the high burden countries of pulmonary tuberculosis in the world. PTB is a major health problem in Sikkim.

The rapid transmission and emergence of TB has made it mandatory for laboratories and health care centres to quickly detect and diagnose MTB from the clinical samples. Exploring newer diagnostic tests for the immediate detection of MTB is the need of the hour. The result of the study may be able to narrow the gap in the knowledge regarding the significance of IGRA in the diagnosis/screening of PTB.

Materials and Methods

Study setting and study population:
A cross-sectional study was performed in a tertiary care central referral hospital, Sikkim from 2015 to 2017. The institutional ethical clearance was obtained prior to the study. Only new clinically suspected cases of PTB, not on any anti-TB drug regimen and providing consent were included in the study.

Sputum smear microscopy:
Two sputum samples; one spot and one early were collected in a sterile, leak proof 5-10ml container and were transported to the Microbiology Tuberculosis laboratory. At the laboratory Auramine-O staining was performed and the slides were examined under a fluorescence microscope to identify sputum positive and sputum negative samples.

IGRA:
For IGRA, 5ml whole blood was collected in BD vacutainer tubes provided by the manufacturer. The Immucheck TB Platinum is based on the principle of IGRA wherein the whole blood is collected from the suspected person in the BD vacutainer tubes and is stimulated against MTB specific antigens in the culture tubes to release IFN-γ which is assayed further.1ml of blood from vacutainer tubes is dispensed in the two culture tubes. Background control culture tube (N), Testing culture tube (T) within 12hours of blood collection.

These culture tubes are then incubated at 37° C for 22±2 hours, after which plasma is harvested and the plasma is then tested for the presence of IFN-γ produced in response to the peptide antigens. The "T" tube contains the peptide antigens specific for MTB viz (ESAT-6) and (CFP-10). The antigens (ESAT-6) and (CFP-10) selected for stimulation are specific fragments that pathogenic MTB have, but BCG vaccine and other Mycobacterium do not have. The patients infected with MTB have specific T-lymphocytes which can identify these antigens and T-lymphocytes will be stimulated to proliferate and release cytokines such as IFN-γ. This released IFN-γ is then further assayed for quantification and checked for TB infection and TB disease.

For interpretations, the IFN-γ level of the "N" tube is subtracted from the IFN-γ level for the "T" tube. The "N" tube adjusts for background, heterophile antibody effects, or non-specific IFN-γ in the blood samples. Further, TB platinum software is used for the interpretation of the results.

Outcome classification:
We defined the reference standards for active TB as a positive chest x-ray interpretation for whom ATT was started. Chest x-ray results were interpreted with no knowledge of IGRA results.

Statistical analysis:
We performed the sensitivity and specificity of the diagnostic tests keeping chest x-ray results as a reference standard. We compared the sensitivity and specificity of fluorescence microscopy and IGRA using McNemars's test. Fisher's exact test was used to determine the p-value. The clinical utility of the diagnostic tests was also determined. For this analysis we considered pre-test probability as the ability of IGRAs in diagnosis of TB before any prior conformation of the existence of the disease in the suspected cases. However, the post-test probability was determined by calculating the efficacy of IGRAs along with the chest x-ray in correctly diagnosing the disease. We performed all the analysis using GraphPad Instat (GraphPad Software Inc., 5755 Oberline drive, San Diego, USA), with the level of significance specified in reference to two tailed, type one error (p-value) less than 0.05.

Results

Study population:
A total of 300 patients fulfilling the inclusion criteria were included in the study. Amongst the total study population 80(26.67%) cases were chest x-ray positive, 50(16.67%) were Fluorescence microscopy positive and 82(27.33%) cases were IGRA positive.

Fluorescence microscopy: When evaluating with the referred standard outcome of chest x-ray results 50(62.5%)

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cases out of 80 chest x-ray diagnosed cases were found to be positive for Fluorescence microscopy.

IGRA results: A total of 82 cases out of 80 chest x-ray diagnosed cases were found to be positive by IGRA. Therefore, IGRA being able to diagnose 2 additional cases than that of chest x-ray. However, an indeterminate result was also found by IGRA due to reduced lymphocyte count in the blood sample. IGRA also proved to be capable of diagnosing TB in 3 children.

Diagnostic performance:
Over all IGRA was found to be 100% (95%CI=0.98-1.00) sensitive and 99.09% (95% CI=0.91-0.99) specific with a positive predictive value of 97.56% (95% CI=0.97-0.99), negative predictive value of 100% (95% CI=0.96-1.00) and an efficacy of 100% in the context of diagnosing active TB cases (P<0.0001); with a likelihood ratio of 41.00. However, FM showed a sensitivity of 88% (95% CI=0.83-0.92), specificity of 100% (95%CI=0.93-1.00), positive predictive value of 100% (95%CI=0.98-1.00) and a negative predictive value of 62.5% (95%CI=0.51-0.73) with an efficacy of 90% (P<0.0001). This result was determined in context to Chest x-ray result.

Further analysis showed that both IGRA and Fluorescence microscopy results were positive in 50 patients and negative in 218 cases, the overall correlation was observed in 268 patients, i.e.; 89.33%. In the remaining 32(10.67%) patients, IGRA results were found to be positive but Fluorescence microscopy negative. Here we also calculated the diagnostic utility of IGRAs in reference to fluorescence microscopy by determining the post-test probability of TB in patients with positive and negative IGRA results. In this investigation, the pre-test probability was defined as the TB prevalence in the study population: 100% in fluorescence microscopy positive and 40% in fluorescence microscopy negative patients. The IGRA result did not alter the probability of disease detection among the fluorescence microscopy positive patients. However, positive IGRA results for fluorescence negative patients increased the probability of case detection from 16.67% to 27.34%, i.e.; an overall increase of 11% cases. A negative IGRA result decreased the probability of TB disease from 83.33% to 72.67%, allowing the least chance of missing the TB disease.

Discussion

In this study, we found that IGRA was positive in 27.34% of the suspected patients, indicating 1/4th of the new suspected cases suffering from the disease, thus implying a prevalence rate that cannot be overlooked. IGRAs positivity was not affected by any justified cause and was found to be 100% sensitive and 99.09% specific in the overall case detection as compared to the gold standard. Studies have reported ~99% specificity eliminating false positive readings, similar to our study.[3],[9],[11],[22] Fluorescence microscopy negative but IGRA positive result, presented an increase in 11% case detection, which is clinically important to avoid any chances of missing the positive cases which may be a probable cause of spreading the TB disease in the society at a very high rate. Although patients with sputum smear-negative TB are less infectious, they are more likely to die during or before diagnosis than patients with smear positive TB, and contributes to TB transmission.[14],[16],[17],[20] Which may also result in the spread of disease in the society and an improper management of the disease. Thus, jeopardizing the TB control Programme.

FM showed a sensitivity of 62.5%, specificity of 100%. Thus, IGRA being more efficient/ accurate than that of Fluorescence microscopy. A lower degree of correlation was observed in the case detection ability of Fluorescence microscopy when compared to that of chest x-ray. This could be interpreted as, in studies it has been shown that the cases where chest x-ray and clinical history are very suggestive of TB but smear are negative, treatment can be started as in context of good sputum collection with induction, three sputum cultures have a sensitivity of 90%, which means that 10% of the active TB cases will be missed.[12],[15]

To have a clinical utility, a diagnostic test result must influence the ability of a person in taking an unbiased decision. While evaluating a TB suspect with negative sputum microscopy result medical officials faces a dilemma of either withholding the treatment or to go with further analysis. IGRAs could be useful in cases with negative results, thus decreasing the probability of a TB disease to an empirical level, where the medical officials would be clear enough to withhold further analysis and treatment. In lieu of the test results obtained in our study negative IGRA results lowered the probability of TB disease by 11%. It has also been reported in a study that IGRAs provide an objective, reproducible result that is unaffected by subjective interpretation.[9],[10] However, only one intermediate result was obtained in our study thus, presenting a considerable amount of error in our study setting. Moreover, the IGRAs results are unaffected by previous BCG vaccination or other environmental mycobacteria.[3] IGRA tests can be available in 24 hrs.[12],[20] Thus, adding an advantage to our study.

Two additional cases were diagnosed by IGRA which were neither Fluorescence microscopy positive nor chest x-ray positive. The possible cause being, to form a granuloma or tubercle, IFN-γ and TNF- alpha (tumor necrosis factor-alpha) are essential in this phase of adaptive immunity. The subjects may or may not be positive for Tuberculin Skin Test or IGRA at this stage and if asymptomatic, they are deemed as having latent infection.[13],[19],[21] The study revealed the advantage of IGRA in diagnosis of childhood

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TB as it could diagnose TB infection in child patients also, which was not diagnosed by Fluorescence microscopy but chest x-ray. As stated that there is no single test that works well in childhood TB.[14],[21] Thus, the pediatricians have to rely on a series of tests to diagnose childhood TB, with no assurance of positive prediction by the tests.

Limitation of our study was that only a small number of suspected TB patients were present as the patients included were only the ones who attended CRH. However, potential cases might have been missed due to fact that the patients might not have attended our hospital, may not have been seriously ill and may be they attended other hospitals or private facilities. Therefore, the clinical findings of our study may also vary depending on the load of the disease in this region. Our findings suggest a role for IGRAs as a tool in investigating TB disease but could not justify its use as a major screening tool as chest x-ray is not an absolute standard means of diagnosing a TB disease and has its own limitations. Using a more reliable screening tool might have helped in the better and more reliable interpretation of the efficacy of IGRAs. Moreover, the efficacy of IGRA was only tested in PTB patients and not Extrapulmonary tuberculosis (EPTB) patients. Thus, the ability of IGRA in diagnosing EPTB cases was not determined which may have proved to be of greater importance[9] or cases of LTBI that may eventually convert into PTB or EPTB was also not determined. Forcing the need for a large metacentric study. However, our findings are consistent with the previous studies performed.

Thus, it can be concluded that a reliable estimate of results was presented by IGRAs in our study settings. A considerable prevalence of TB infection in the suspected patients was observed in patients attending CRH, Sikkim. This assay should be considered while diagnosing TB cases to eliminate the possibility of doubt in TB negative patients and to assert other tests for diagnosing TB infection in IGRA positive tests, avoiding any chance of missing a potential TB case. Furthermore, IGRAs could be used as a tool in diagnosing TB in children as it is difficult to obtain sputum samples from children. Thus, effortlessly combating the TB control Programme.

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