INTERFERON GAMMA RELEASE ASSAY AMONG TUBERCULOSIS CULTURE POSITIVE AND NEGATIVE CASES TO DETERMINE CUT OFF VALUE


Pakistan Air Force Hospital, Faisal Base, Karachi Pakistan, *National Institute of Health, Islamabad Pakistan, **Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, ***Combined Military Hospital Malir/National University of Medical Sciences (NUMS) Pakistan, ****Combined Military Hospital Sibi Pakistan

ABSTRACT

Objective: To determine cut off value of Interferon Gamma Release Assay (IGRA) among Tuberculosis (TB) suspected patients and to compare median Interferon Gamma Release Assay value for Tuberculosis culture positive and culture negative patients.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, National University of Medical Sciences, from Oct 2015 to Apr 2016.

Methodology: The study was conducted on patients with suspected tuberculosis. Quantiferon Tuberculosis Gold that has an important role in the immune response to Tuberculosis was performed by Enzyme Linked Immunosorbent Assay method and specimens were cultured on Mycobacterium Growth Indicator Tube 960 System (MGIT 960 system). Quantiferon Tuberculosis Gold values obtained by Enzyme Linked Immunosorbent Assay method were used to determine the sensitivity and specificity of this test.

Results: A total of 240 suspected isolates of tuberculosis during the study period were subjected to culture using MGIT 960 system, as per recommended protocol for the method. These samples were also subjected to Quantiferon TB Gold (QTBG) to measure Interferon Gamma Release Assay release. Sensitivity and specificity of IGRA was determined keeping tuberculosis culture as gold standard for this study.

Conclusion: Sensitivity and specificity values showed a significant p-value <0.001 with active tuberculosis. In TB endemic areas like Pakistan, new cutoff value must be kept in mind while interpreting results of IGRA.

Keywords: Enzyme linked immunosorbent assay, Interferon gamma release assay, Mycobacterium growth indicator tube 960 system, Quantiferon TB Gold.

INTRODUCTION

Among the infectious diseases, Tuberculosis (TB) remains a significant cause of global morbidity and mortality. Approximately 9.6 million new cases of TB were noted globally in 2015 with 1.5 million TB related deaths1. A newly infected individual can become ill from TB within weeks to months, but most infected individuals remain well2. Pakistan is one of high tuberculosis burden countries in the world3.

Interferon gamma release assays have been introduced lately for diagnosis of latent TB. Quantiferon TB Gold assay is an in vitro diagnostic test utilizing ESAT-6, CFP-10 and TB-7.7 proteins to stimulate cells in whole blood4. A recent study has shown that Interferon gamma release assay (IGRA) is supposed to have better specificity than tuberculin skin test5. IGRA are more accurate than tuberculin skin test even in children due to better specificity6. Commercially available IGRA measure interferon gamma release with an immunoenzymatic assay and results are reported as per specific cut off value7,8. A study conducted in United States revealed that as compared to tuberculin skin test QTBG assay is more specific especially in previously vaccinated individuals9.

A Syrian study revealed that IGRA was 89.9% sensitive in pulmonary TB patient10. Simple methods enhancing detection of Mycobacterium tuberculosis are needed for early diagnosis and effective treatment of pulmonary tuberculosis10.
If IGRA and real time polymerase chain reaction are negative with positive culture, there is likelihood that 60% cases may be due to mycobacterium other than tuberculosis. These cases may require further identification tests on Lowen-Stein Jensen slopes. People residing in high TB burden area with one or more risk factors may be harboring latent tuberculosis infection and IGRA positive. IGRAs in addition to tuberculin skin test can be used for screening of TB infection10.

METHODOLOGY

This study was carried out at department of Microbiology, Armed Forces Institute of Pathology, National University of Medical Sciences from October 2015 through April 2016. Permission was sought from Institutional Ethical Committee; individual written consent was taken from each case. Brief history and demographics were recorded in each case. A total of 240 suspected isolates of tuberculosis were selected by non-probability consecutive sampling. Requisite samples were taken for TB culture including respiratory specimens, CSF, tissues, body fluids and blood for QTBG assay. Sputum was the commonest specimen used for the diagnosis of pulmonary tuberculosis. If sputum cannot be obtained due to any reason, then inhalation of hypertonic saline or postural drainage may be beneficial to obtain the sputum specimen. Bronchoalveolar lavage (BAL), tracheal secretions, gastric aspirates (swallowing of AFB during night), and biopsies of respective suspected sites may also be used for diagnosis. Most likely because of risk of intermittent shedding of bacilli in urine, the yield of urine examination a smear and culture for detecting the tubercle bacillus is quite low.

IGRA was performed using the QTBG assay (Cellestis, QIAGEN Company (Australia). This test is based on the principle (fig-1) that a cytokine known as (interferon-gamma IFN-γ) is produced by activated T-lymphocytes in patients of tuberculosis (TB). It has an important role in the immune response to TB. Mixtures of synthetic peptides representing two MTB proteins, ESAT-6 and CFP-10 are used in this test as antigens. Two collection tubes for each patient (TB Antigen and Nil Control tubes) were used, each having 1 ml blood. Volume of blood drawn into tube can alter results13. The tubes were incubated for 16 to 24 hours at 37°C ± 1°C. After incubation, harvesting of plasma was facilitated by centrifuging the tubes for 15 minutes at 2000 RCF (g). Standard was reconstituted with concentration of 8 IU/ml. Conjugate 100X in freeze dried form was reconstituted with 0.3 ml of distilled water. Working strength conjugate was prepared by diluting required amount in Green Diluent. Freshly prepared working strength conjugate 50 μl measuring was added to enzyme linked immunosorbent assay (ELISA) wells followed an equal amount of test plasma sample. Mixture was incubated at room temperature for two hours. During incubation one part of buffer concentrate was washed with 19 parts of distilled water. Wells were washed with 400 μl of working strength buffer for at least 6 cycles. Enzyme substrate solution measuring 100 μl was added to each well; plates were incubated at room temperature for 30 minutes. Then 50 μL of enzyme stopping solution was added to each well and mixed, optical density of each well was measured using a micro plate reader fitted with a 450 nm filter and a reference filter of 620 nm to 650 nm optical density. Present cut off value is 0.35 iu/ml. In case of tuberculous patient, white blood cells released IFN-γ in response to contact with the TB antigens.

Sputum samples for TB culture were digested and decontaminated as per standard protocol. The samples were then cultured using commercially available MGIT 960 automated TB culture system (Becton Dickinson, USA). MGIT is a non radiometric automated system for detection of MTB based on the principle of oxygen quenching having an advantage of early detection, as the machine monitors and reads the tubes placed inside machine hourly. As per the manufacturer’s recommendations, all positive MGIT 960 tubes were subjected to Ziehl Neelsen (ZN) staining for the confirmation of presence of AFB,
Tuberculosis Culture Positive And Negative Cases


(mycobacteria appear as pink straight or slightly curved rods in ZN staining, sometimes giving beaded appearance against a blue or light blue background) and sub cultured on 5% Sheep Blood Agar (Oxoid, UK) to check for contaminants. Commercial immunochromatographic test (Becton Dickinson, USA) was used to differentiate between Mycobacterium tuberculosis (MTB) complex and MOTT on BACTEC MGIT 960 system. Other samples like fluids were directly used for culture on MGIT 960. The H37Rv strain (ATCC 27294) was used for QC of testing.

The data obtained were entered in SPSS (version 17). Shapiro-wilk test was applied to check normality. For qualitative data frequency and percentage were calculated. For quantitative data mean ± SD was calculated. Median and IQR were calculated for non-normal variables. Independent t-test was applied to compare age between positive and negative culture values and Mann whitney U-test for comparison of IGRA values. Chi-square test is applied on gender. ROC curve to find out cutoff value of IGRA with sensitivity and specificity (area under the curve) was presented, the level of significance (p ≤0.05) was considered significant.

RESULTS

A total of 240 suspected isolates of tuberculosis during the study period were subjected to culture using MGIT 960 system as per recommended protocol for the method. Specimens were also taken to measure IGRA values for same patients.

Median IGRA value was 0.12 with IQR 0.33 for culture negative and 0.7200 with IQR 0.36 for culture positive cases with p-value of <0.001 which was significant (table).

Cut off value was 0.455 with sensitivity of 91.7% and specificity of 96.7%. AUC was 99.2% with p-value <0.001, which was significant (fig-2).

Table: Comparison of age, gender & IGRA values between the groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Culture Negative (n=120)</th>
<th>Culture Positive (n=120)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.62 ± 17.31</td>
<td>39.52 ± 17.13</td>
<td>0.621</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28 (23.3%)</td>
<td>27 (22.5%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92 (76.7%)</td>
<td>93 (77.5%)</td>
<td></td>
</tr>
<tr>
<td>IGRA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.12 (0.33)</td>
<td>0.72 (0.36)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Average age of culture negative cases was 40.62 ± 17.31 years with minimum age of 3 years and maximum age of 85 years while in culture positive cases average age was 39.52 ± 17.13 years with minimum age of 13 years and maximum age of 80 years. Both the groups are similar with respect to age with insignificant difference (p=0.621).

Among culture negative cases, 28 (23.3%) were females while 92 (76.7%) were males. Among culture positive cases, 27 (22.5%) cases were females while 93 (77.5%) were males.
Gender distribution was similar between both the groups with insignificant difference ($p=0.878$) table-I.

New cutoff value 0.455 IU/ml was defined in our study which was higher than 0.35 IU/ml which was defined by manufacturer and it is statistically significant. New cutoff value must be kept in mind while interpreting results of IGRA. Any person whose IGRA value is more than 0.455 IU/ml is more likely to develop active TB in future.

**DISCUSSION**

Tuberculosis is also known as a disease of poverty, affecting mainly low socioeconomic group, those who live in ill-ventilated and crowded places, and those who are ill-fitted to combat tuberculosis in terms of their immune status or whose nutritious level is so low that they are easily prone to tuberculosis11-13.

Tuberculosis is one of leading causes of mortality among infectious diseases. Despite the great advancements in the field of medicine, tuberculosis remains one of the biggest infectious diseases in developing countries like Pakistan. Children are more vulnerable to develop life threatening TB as compared to adults14. The key to control the spread of this disease in community is early diagnosis, effective treatment and strict isolation of the infectious patients. Multidrug resistant tuberculosis (MDR-TB) is a real challenge in Pakistan where diagnostic as well as DST facilities for MDR-TB are sparse along with poor infection control practices.

The prevalence definitely increases in a developing country like ours due to scarcity of proper diagnostic facilities especially MTB culture and molecular tests. There is a dire need to find some alternatives for the huge burden of suspected cases. The test has been more pertinent for non endemic TB region, however we wanted to evaluate this test in a different manner for redefining its value in an endemic country. This study was conducted for evaluating a new cut off value by correlating culture and QTBG assay values in suspected cases of TB.

A number of studies have been conducted worldwide to determine the sensitivity and specificity for IGRA. Our study focused on determining the mean cut off value for IGRA for culture positive and culture negative cases. In our study a new cut off value was defined which was higher than value defined by manufacturer with sensitivity and specificity of 91.7% and 96.7% respectively comparable with a meta analysis which showed the specificity to be in a range of 98-100%.15.

A study published in European Respiratory Journal showed IGRA to have comparative advantage over Mantoux test in detecting latent TB infection16. People living in high burden area with one or more risk factors may be harboring latent tuberculosis infection and have interferon gamma release assay positive. IGRA represents an indirect marker of MTB exposure17. Neither tuberculin skin test nor QTBG can differentiate between active disease and latent infection.

A study conducted in USA revealed that as compared to tuberculin skin test QTBG assay is more specific especially in previously vaccinated individuals18.

**CONCLUSION**

A new cutoff value 0.455 was defined in our study higher than manufacturer value 0.35, it was statistically significant. In TB endemic areas like Pakistan, new cutoff value must be kept in mind while interpreting results of IGRA. Person with IGRA value more than 0.45 IU/ml is more likely to develop active TB in future. The advantages of this test included: Results were available within 24 hours, results were not affected by prior BCG vaccination, and the test required a single visit of patient to draw blood sample.

**CONFLICT OF INTEREST**

This study has no conflict of interest to declare by any author.

**REFERENCES**


