Evaluation of pulmonary tuberculosis with clinical and computed tomography aspect via newer diagnostic molecular modalities: An original research

Dr. Rahilla Tabassum  
Senior resident, Department of Microbiology, Government Medical College, Rajouri, Jammu and Kashmir, India.  
Email: drrahilla10@gmail.com

Dr. Izna  
Senior Resident, Department of Microbiology, Government Medical College, Rajouri, Jammu and Kashmir, India.  
Email: choudharyizna76@gmail.com

Dr. Arshad Ahmed  
Assistant Professor, Department of Radiology, Government Medical College, Rajouri, Jammu and Kashmir, India.  
Corresponding Author email: drarshad14@gmail.com

Dr. Ishtyaq Ahmed  
Demonstrator, Department of Physiology, Government Medical College, Rajouri, Jammu and Kashmir, India.  
Email: Ishtyaq106@gmail.com

Dr. Mushtaq Ahmed  
Assistant Professor, Department of Surgery, Government Medical College, Rajouri, Jammu and Kashmir, India.  
Email: mushtaqchowdhary53@gmail.com

Dr. Asim Naik  
Senior Resident, Department of Ophthalmology, Government Medical College, Rajouri, Jammu and Kashmir, India.

Abstract---Aim: The purpose of the present research was to assess the pulmonary tuberculosis cases, its clinical as well as computed tomographic (CT) features with the help of newer diagnostic molecular techniques. Methodology: A total of 100 clinically diagnosed TB patients were incorporated in this study. Sputum were gathered for...
smear microscopy, culture (Lowenstein–Jensen medium) and PCR testing. The sensitivity of smear and PCR were compared to that of culture considering as gold standard. 50 of 100 patients were positive on smear microscopy. Results: 63 specimens yielded the growth of Mycobacterium tuberculosis on Lowenstein-Jensen's medium and PCR detected the presence of MTB specific gene in 77 specimens with CT scans positive in 51 cases. In clinical diagnosis of tuberculosis, molecular methods are probably a useful adjunct certainly in smear negative paucibacillary cases. Conclusion: PCR is specific, rapid, more sensitive but expensive technique. The sensitivity of PCR was remarkably high when compared to smear and culture as well as CT scan.

**Keywords**—Mycobacterium tuberculosis, Tuberculosis, molecular diagnosis, PCR.

**Introduction**

Tuberculosis (TB) is a global health concern for both developing and developed countries and has recently become more complex due to persistence in aging populations and the rise of drug-resistant strains. In clinical practice, rapid TB diagnosis can be difficult, and early pulmonary TB detection continues to be challenging for clinicians. Prompt diagnosis of active pulmonary TB is a priority for TB control, both for treating the individual and for public health intervention to reduce further spread in the community. Chest X-ray is useful but is not specific for diagnosing pulmonary TB. Moreover, TB can present with symptoms and atypical radiologic findings that are indistinguishable from those of community-acquired pneumonia. As a result, it is not unusual for clinicians to prescribe a number of courses of antibiotics for pneumonia before the pulmonary TB is correctly diagnosed. Therefore, an acid-fast bacilli (AFB) smear and bacteriological culture tests should be performed for patients with symptoms that are compatible with or suggestive of TB. However, mycobacterial culture, which has the highest sensitivity for diagnosing and confirming active TB, requires 2 to 6 weeks for interpretation. Although sputum smear microscopy is a rapid, simple, and inexpensive tool for diagnosing pulmonary TB, it has low and variable sensitivity. Recently, non-molecular and molecular assays have been developed for early detection of active TB with or without drug resistance detection. The diagnosis of TB is suspected from a combination of context, symptoms, clinical signs and investigations. Pulmonary TB refers to any bacteriologically confirmed or clinically-diagnosed case of TB that involves the lung parenchyma or the tracheobronchial tree based on the revised previous standard case definitions for TB by the World Health Organization (WHO) in 2013. Imaging methods have long been used to aid in TB diagnosis. Despite its low specificity, chest radiography is still an extremely valuable technique used in the initial management of patients with respiratory symptoms. Moreover, chest radiography is a valuable complement to physical examination, as it can detect multiple clinical changes and it is essential in differential diagnosis. Chest computed tomography (CT) is more sensitive than radiography in detecting initial clinical changes. In addition, CT can distinguish active lesions from residual lesions in
most cases, accurately assess the extent of the disease, and determine a diagnostic standard based on the major changes observed. Therefore, although this method is not highly specific for TB diagnosis, CT adds diagnostic information and is a particularly valuable method for patients with suspected TB and negative AARB test results, allowing adequate therapeutic decision-making while waiting for sputum culture results to confirm TB. Magnetic resonance imaging (MRI) could be a new diagnostic tool, which may fill the missing gaps in the radiological diagnosis of pulmonary TB, its role being well recognized in extrapulmonary disease assessment, such as neurological and spinal involvement. MRI emerged as an alternative – radiation-free method for the evaluation of lung diseases – after chest radiography and computed tomography (CT). The recent technical advances have helped MRI to overcome its main limitations (low proton density and low signal-to-noise ratio) for lung characterization. From a historical perspective, Gallium-67-citrate ([67Ga]Gacitrate) was one of the first radioisotope workhorses used in the management of TB and had been employed in the clinical practise to give an indication of disease activity, response assessment, and disease extent including the ability to differentiate infection from Mycobacterium tuberculosis (MTb) from non-MTb lesions. 2-deoxy-2-[18F]fluoro-D-glucose (2-[18F]FDG) has been the most widely studied PET radiopharmaceutical in TB, with a growing interest in Gallium-68-based radiopharmaceuticals.

Aim of the present study

The purpose of the present research was to assess the pulmonary tuberculosis cases, its clinical as well as computed tomographic (CT) features with the help of newer diagnostic molecular techniques.

Methodology

A sum of 100 samples received at our institution with clinical suspicion of tuberculosis were included in the study. The samples collected were processed for smear examination, culture on Lowensten-Jensen’s media and detection of MTB specific gene using PCR. The results of all different diagnostics were compared and analysed. The sensitivity of PCR test, smear microscopy was evaluated considering culture as gold standard. Zeihl-Nielsen (ZN) staining was done on these smears using standard techniques and observed for the presence of acid fast bacilli (AFB). AFB was graded using IUALTD recommendations. All the specimens were further subjected to digestion and decontamination using standard N-acetyl L-cysteine – NaoH (NALC-NAOH) method. Mycobacterial isolates obtained were subjected to MPT64Ag ICT test for confirmation of isolates as MTBC. DNA extraction and amplification of 225bp of M.tuberculosis was done by using commercially available HELINITM pure fast bacterial genomic DNA minispin prep kit and HELINI MTB PCR kit. DNA amplification by PCR was performed with a total reaction volume of 20µl by using Biorad PCR systems. After final extension the samples were immediately kept at -20°. PCR products were using 1% agarose gel in 0.5X TAE buffer containing ethidium bromide at 10µg/ml concentration and the samples showing the presence of band at 225bp under gel doc were considered as positive for M. tuberculosis. Statistical analysis
such as sensitivity and diagnostic accuracy for various diagnostic tests was calculated in this study.

Results

Of 100 samples included, 50 were smear positive and 50 were smear negative. Among 100 patients, 59 (59%) of the patients were males and 41 (41%) were females. The mean age of subjects among males and females were 44.63 and 40.15. Considering 19.28 as standard deviation for males and 22.32 for females, overall age of the total subjects was 42.79±20.59. A total of 100 samples were subjected to microscopy, culture on LJ medium and PCR. Sensitivity of PCR test with two different tests was compared for pulmonary and extrapulmonary samples. The PCR test gave a higher sensitivity compared to ZN smear and LJ medium in both pulmonary and extra pulmonary samples. The diagnostic accuracy of PCR was calculated by considering culture as gold standard and the diagnostic accuracy of PCR is 74%. The sensitivity of PCR was better than CT scan (51%). (Table 1&2)

Discussion

World health organization on the motto of end TB strategy and with the goal of reducing the spread of TB has been recommending for the development of newer and reliable TB diagnostic tools which would be more sensitive and simpler. Scientists and researchers around the world have been working on these strategies of ending TB and reducing the spread of infection. Efforts for development of rapid and low-cost tests with high sensitivity and specificity which can be used in limited resource settings as a point of care diagnostics are still on its way with the goal of decreasing TB. Evidence based diagnosis like smear microscopy is very much needed for the efficient diagnosis of TB. But it has its own limitations such as low sensitivity. Hence, this is the primary disadvantage and there is a need for developing new tools to diagnose tuberculosis. Sensitivity of Microscopy in our study is 50%, was moderately high than compared to other studies. This might be due to in appropriate selection of samples where among a total of 100 samples, 50 smear positive and 50 smear negative samples. A wide range of smear positivity between 0-75% has been reported in earlier studies. Prompt and accurate diagnosis of tuberculosis is still a dilemma in developing countries where LJ culture is still used as the gold standard for its diagnosis. The culture is time consuming (4-8 weeks) therefore, evidences like histology/cytology along with clinical evaluation are still being used to treat the patient with a full course of anti-tubercular treatment. In the present study, the sensitivity and yield of culture positivity was almost equivalent when compared with the smear positivity. Diagnostic techniques based on amplification have the potential to increase the sensitivity for detecting mycobacterium which can also reduce the turnaround time that is usually necessary to isolate and to identify these organisms using biochemical reactions. A sensitivity of 77% for PCR test was seen in our study, this is concordance with a study who reported a sensitivity of 74.4%. Various studies showed varied results for sensitivity for PCR. In a study conducted by Muhammad Kashif Munir et al., sensitivity of 92% was reported. The sensitivity of PCR was remarkably high when compared to smear and culture in this study and PCR took much shorter time (1-3 days) as compared to culture
(6-8 weeks). In a pooled analysis of 125 studies\textsuperscript{26}, the overall sensitivity of PCR was 85\%. The results of this study states that smear is cheap and rapid method of detecting mycobacterium tuberculosis but it has a very low sensitivity. Culture is more sensitive but it takes a longer time to give results while PCR is specific, rapid, more sensitive but expensive technique, and can be used in difficult cases where diagnosis become a challenge.

**Conclusion**

Diagnosis by culture, even though being gold standard, relatively specific and sensitive in comparison with smear microscopy but slow and time taking process. A quicker and yet accurate diagnosis of Mycobacterium tuberculosis is pivotal in the management of TB. PCR is specific, rapid, more sensitive but expensive technique. The sensitivity of PCR was remarkably high when compared to smear and culture as well as CT scan.

**References**

12. Negi S.S., Khan S.F.B., Gupta S., Pasha S.T., Kare S., Lal S. Comparison of the conventional diagnostic modalities, BACTEC culture and polymerase


