Section: Miscellaneous

Original Research Article

REVOLUTIONIZING TB DIAGNOSIS: A COMPARATIVE STUDY OF ZIEHL-NEELSON STAINING VS. GENEXPERT MTB/RIF

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ABSTRACT

Background: Tuberculosis (TB) is the world's serious public health issue with a burden of estimated 8.7 million new cases and 1.4 million deaths annually. A significant problem in isolated and rural areas is the diagnosis and treatment of tuberculosis. **Aims & Objectives:** To evaluate the diagnostic accuracy of the GeneXpert MTB/RIF assay for the detection of M. tuberculosis along with the sensitivity & resistance for the rifampicin in pulmonary and extra pulmonary specimens and to compare it with conventional techniques. To access diagnostic usefulness of Gene Xpert MTB/RIF assay technique in management of tuberculosis.
Material & Methods: A cross sectional Study was conducted at tertiary care hospital Ahmedabad from 1 August 2021 to 30 November 2021 with enrollment of 616 total samples. The samples were subjected to Ziehl-Neelsen (ZN) staining, GeneXpert MTB/RIF (Cepheid, Sunnyvale, US) assay. **Results:** Total no. of samples were 616 included both pulmonary & extra pulmonary. Out of total samples, 516 samples were pulmonary (sputum, Broncho-alveolar lavage & gastric aspiration) & 100 samples were extra pulmonary (pleural fluid, cerebrospinal fluid &ascitic fluid). Out of 516 pulmonary samples 256 (49.21%) had shown Mycobacterium Tuberculosis detected by Gene Xpert. Out of this 256 samples, 84 (32.81%) samples were found AFB smear negative & 172 (67.18%) samples were AFB smear positive. Out of 516 Pulmonary samples 260 (50.38%) had not shown Mycobacterium Tuberculosis by Gene Xpert. Out of this 260 samples, 252 (96.92%) samples were AFB smear negative & 08 (3.07%) samples were found AFB smear positive. Out of total 256 positive samples, 232 (90.90%) samples were sensitive for rifampicin and 24(9.37%) samples shown resistance.
Conclusion: Gene Xpert is better tool for identification of mycobacterium tuberculosis and rifampicin sensitivity from the same sample within 2 hrs. Compared to microscopy it detects 34% more no. of cases with higher specificity.
Keywords: Tuberculosis (TB),Multi drug resistance (MDR),Extended drug resistance (XDR).

INTRODUCTION

Mycobacterium tuberculosis, often known as M. tuberculosis, is the organism that causes tuberculosis (TB). With an estimated 8.7 million new cases and 1.4 million deaths annually, tuberculosis (TB) continues to be the world's most serious public health issue.[1] In India, the estimated prevalence of

tuberculosis (TB) is higher in tribes (29.9%) than in non-tribal populations (21.4%).[2]A significant problem in isolated and rural areas is the diagnosis and treatment of tuberculosis.Conventional diagnosis procedures of today necessitate frequent hospital visits and well-established laboratories. Patient noncompliance has led to the emergence of Multi drug resistance (MDR)and Extended drug resistance (XDR) TB, which have greater death rates. Resistance to the two most effective anti-TB medications, isoniazid (INH) and rifampicin (RIF), is referred to as MDR-TB. The most crucial medication for treating tuberculosis is RIF. Extended treatment regimens involving comparatively more toxic second-line medications are necessary for Rifampicin resistance-MTB infection.[3]Since MDR- and XDR-TB are becoming more common and have a significant risk of transmission from person to person, it is critical to diagnose patients quickly and use antitubercular drugs sensibly.[4]

As the "gold standard" for detecting mycobacterium tuberculosis sensitivity, culture is a slow process that can take up to 4-6 weeks to complete and requires sophisticated biosafety laboratory infrastructures with skilled human resource, which is largely not available in rural settings.[5]The GeneXpert MTB/RIF assay is a new integrated diagnostic tool that performs rapid diagnosis of TB as well as detection of Rifampicin resistance in unprocessed sputum samples.[6,7]The test can be performed in ordinary laboratory conditions without isolation of MTB and is currently a suitable technique for rural settings. So, the present study wasaimed at GeneXpertMTB/RIF assay based rapid diagnosis of TB and Rifampicin resistance detection at Tertiary care hospital, Ahmedabad.

Aims & Objectives

- To evaluate the diagnostic accuracy of the XpertMTB/RIF assay for the detection of M. tuberculosis along with the sensitivity & resistance for the rifampicin in pulmonary and extrapulmonary specimens and to compare it with conventional techniques.
- To access diagnostic usefulness of Gene Xpert MTB/RIF assay technique in management of tuberculosis.

MATERIAL AND METHODS

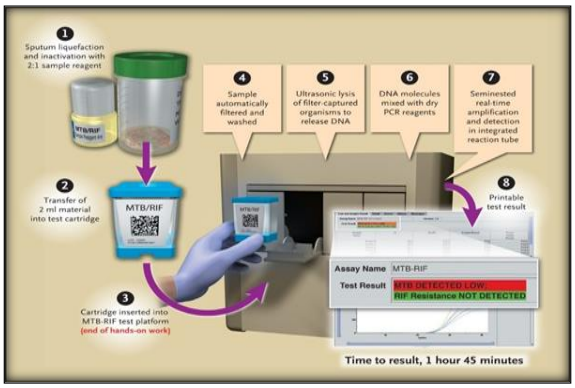
A cross sectional Study was conducted among total no. of samples were 616 included both pulmonary & extra pulmonary, were received in the Tuberculosis lab, at tertiary care hospital Ahmedabad under the National Tuberculosis Elimination Programme(NTEP) during the Period, form 1 August 2021 to 30 November 2021.The samples were subjected to Ziehl-Neelsen (ZN) staining, GeneXpert MTB/RIF (Cepheid, Sunnyvale, US) assay.

GeneXpert MTB / RIF principle

The Xpert MTB/RIF is an automated and rapid test based on nestedreal-time PCR assay and molecular beacon technology for MTB detection and resistance to rifampicin(i.e. mutation of the rpoB gene) in less than 2 hours.[8]Total internal control of reagents system – No separate external positive or negative controls required. Integrated ultrasonic lysis of cells for release of DNA with Automated data analysis and results interpretation.

Processing of samples

Gene Xpert MTB/RIF assay: samples were processed directly from Xpert MTB/RIF test according to manufacturer's protocol. Sample reagent was added in a 2:1 ratio to unprocessed samples in 15 ml falcon tube and the tube was manually agitated (or Vortex) twice during a 15-minute incubation period at room temperature. Then 2 ml of the prepared sample was transferred to the test cartridge by a sterile disposable pipette. Cartridges were loaded into the Gene Xpert machine. The interpretation of data from MTB/RIF tests was software based and not user dependent.



Data entry and analysis: Data were collected by using semi structured Proforma. Data were entered in MS excel sheet 2016 and analyzed by using SPSS software version 26. Qualitative data were described as a Frequency and percentages.

RESULTS

Total no. of samples were 616 included both pulmonary & extra pulmonary. Out of total samples, 516 samples were pulmonary (sputum, Broncho-alveolar lavage & gastric aspiration) & 100 samples were extra pulmonary (pleural fluid, cerebrospinal fluid &ascitic fluid).Out of 516 pulmonary samples 256 (49.21%) had shown Mycobacterium Tuberculosis detected by Gene Xpert. Out of this 256 samples, 84 (32.81%) samples were found AFB smear negative & 172 (67.18%) samples were AFB smear positive. Out of 516 Pulmonary samples 260 (50.38%) had not shown Mycobacterium Tuberculosis by Gene Xpert. Out of this 260 samples, 252 (96.92%) samples were AFB smear negative & 08 (3.07%) samples were found AFB smear positive. Out of total 256 positive

samples,232 (90.90%) samples were sensitive for rifampicin and 24(9.37%) samples shown resistance.

Table 1: Overall results of Gene Xpert

Results	Pulmonary	Extra pulmonary	Total
No. of samples	516(83.76%)	100(16.23%)	616(100%)
MTB Detected	256 (49.21%)	08 (8%)	264(42.85%)
Rifampicin resistance Detected	246 (9.37%)	0 (0%)	24(9.09%)

Table 2: Comparative results of ZN smear and Gene Xpert in pulmonary samples (n=516)

Gene Xpert Findings		
Total no. of samples-516(100%)	**ZN smear positive-180(34.88%)**	**ZN smear negative-336(65.11%)**
Positive-256(49.61%)	172 (67.18%)	84 (32.81%)
Negative-260(50.38%)	08 (3.07%)	252 (96.92%)

Table 3: Comparative results of ZN smear and GeneXpert in extra-pulmonary samples (n=100)

GeneXpert Findings		
Total No. of Samples-100(100%)	**ZN smear positive-00(00%)**	**ZN smear negative-100(100%)**
Positive-08(8%)	0 (0%)	08 (100%)
Negative-92(92%)	0 (0%)	92 (100%)

DISCUSSION

In this study, we have evaluated the diagnostic accuracy of Xpert MTB/RIF assay both for pulmonary and EPTB cases. Out of 616 samples 264 (42.85%) were shown MTB detected by Gene Xpert. In a similar study from six tribal dominated villages of four districts, namely Balangir, Dhenkanal, Kandhamal and Mayurbhanj of Odisha, out of 126 chest symptomatic, TB was detected in 35 (27.77%) subjects.[9] Out of this 264 samples, 92(34%) samples were smear negative & 172 (66%) samples were smear positive. Here, the Xpert MTB/RIF assay diagnose an additional 34% case along with an added advantage of much lower turnaround time.

In present study, out of 516 Pulmonary samples 256(49.21%) had shown Mycobacterium Tuberculosis detected by Gene Xpert. Out of this 256 samples, 84 (32.81%) samples were found AFB smear negative & 172 (67.18%) samples were AFB smear positive. This indicates superior sensitivity of Xpert MTB/RIF assay over ZN smears. Similar observations have been reported in studies by Scott et al (2011).[10]

In the current study, the combined sensitivity of Xpert MTB/RIF assay was 55%. A systematic review and meta-analyses conducted by Denkingeret al showed that Xpert MTB/RIF assay has a sensitivity ranging from 50% to 100%.[11] More recently, Penzet al,[12]reviewed 36 studies in their meta-analyses and confirmed Xpert MTB/RIF assay pooled sensitivity of 87% that is higher to our study.[10]However, the sensitivity of Xpert MTB/RIF assay in the current study is lower than what was found in similar study by Ligthelmet al (sensitivity, 96.7%).[13]

CONCLUSION

GeneXpert is better tool for identification of mycobacterium tuberculosis and rifampicin sensitivity from the same sample within 2 hrs. Compared to rapid method microscopy it detects 34% more no. of cases with higher specificity.

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