

Yield of Xpert MTB/RIF Assay in Extra Pulmonary specimens of patients at tertiary care hospital

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MM NS conceived idea, MM MIM ZA drafted the study, MM NS AS collected data, MM MIM did statistics analysis and interpretation, MIM AC critical review manuscript, All approved final version to be published

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The Authors declares that there is no conflict of interest.

Abstract

Background: Tuberculosis (TB) is the world's leading cause of infectious disease that leads to death and now ranked above AIDS. It is caused by bacteria, Mycobacterium tuberculosis. According to WHO in 2017, 10.4 million people became victim of this infection and 1.8 million of them were died due to this deadly infection. Although TB most commonly infect lungs (pulmonary TB), yet it can spread to sites other than lungs during latent phase of the disease and called as extra-pulmonary Tuberculosis (EPTB). Newer diagnostic techniques like nucleic acid amplification are exceptional for their sensitivity and specificity for detection of MTB in EPTB. One of these latest techniques is GeneXpert MTB/RIF assay.

Objective: Objective of the present study was to observe yield of Xpert MTB/RIF assay in the samples of Extra-pulmonary TB Patients presented at Lahore General Hospital Lahore Pakistan.

Methodology: The specimens of the patients, who are clinically suspected as having TB other than lungs i.e. Pleural effusions, lymph node biopsies, pericardial fluids, ascetic fluids, Cerebrospinal Fluids, urine, joints fluid, bone marrow aspirates, gastric aspirates, stool and skin scrapings were included in the present study. Specimens of pulmonary tuberculosis like Sputum, Broncho-alveolar lavage and blood were excluded from the study. All samples were processed according to standard operating procedures and Gene Xpert was done.

Results: Total 339 samples of extra-pulmonary TB (EP-TB) were collected and processed. Out of these 339 samples, MTB detected in 35 (10.32%) EP samples. Most of these samples were collected from pulmonology and pediatrics departments. Most common sample that was received was gastric aspirates.

Conclusion: Yield of MTB RIF assay was low in our study, though GeneXpert MTB/RIF assay is a very sensitive nucleic acid amplification tests but clinically this test is over-prescribed.

Key Words: MTB/RIF Assay; Extra-pulmonary Tuberculosis; Lahore; Pakistan

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Introduction

Tuberculosis (TB) is the world's leading cause of infectious disease that leads to death and now ranked above AIDS. It is caused by bacteria, Mycobacterium tuberculosis. According to WHO in 2017, 10.4 million people became victim of this

infection and 1.8 million of them were died due to this deadly infection. Adding to this, in 2017 globally 490,000 new cases of Multidrug resistant TB (MDR-TB) were diagnosed, among them 100,000 cases were having rifampicin resistance.¹ MDR-TB is defined, as M. tuberculosis causing TB is resistant to at least

rifampicin and isoniazid, the two first line drugs of TB.² Most of the rifampicin resistant strains are also resistant to isoniazid, as it is used as surrogate marker for detection of multi drug resistance in TB, so we can label these MTB strains as MDR.³

350 cases of TB per 100,000 populations are testified in Pakistan. Pakistan is categorized 5th, amongst 22 high load TB countries. Although TB most commonly infect lungs (pulmonary TB), yet it can spread to sites other than lungs during latent phase of the disease and called as extra-pulmonary Tuberculosis (EP-TB). Worldwide, 10% of extra-pulmonary TB cases are testified every year. EP-TB is often leads to high mortality and morbidity outcome due to its nonspecific and subclinical presentation. It is often difficult to diagnose because the number of AFB is often less in samples of EP-TB. Moreover, these specimens also required some invasive procedures and it is difficult to get multiple samples.^{4,5}

The conventional methods e.g Ziehl-Neelsen staining (ZN) and Culture on Lowenstein-Jensen (LJ) medium have low sensitivity 45-80 % and time requiring diagnostic method, moreover culture of AFB also required proper infra-structure of biosafety lab of level III, these requirements created significant delay in

diagnosing and delays reporting time of AFB.⁶

Newer diagnostic techniques like nucleic acid amplification are exceptional for their sensitivity and specificity. One of these latest techniques is Gene Xpert MTB/RIF assay. It is a rapid, simple, specific and sensitive, fully automated hemi-nested real-time PCR, which amplify and detect Mycobacterium tuberculosis DNA and rpo B gene, which is responsible for rifampin RMP resistance. Hence, we can diagnose MDR TB in less than 2 hours by this technique. The test is done in fully automated disposable plastic cartridge with all required reagents (Cepheid, Sunnyvale, CA).⁷ It is a closed system, that does not require any expertise and there is less chances of biohazard and contamination by this system. It is recommended by WHO, in 2010 as a replacement of conventional techniques for quick detection of MTB in extra-pulmonary samples.⁸

Our study was conducted with the aim to observe the yield of MTB/RIF assay in the specimens of extra-pulmonary TB obtained from the patients presented in Lahore General Hospital. This will help the clinician to reach their final diagnosis by this simple test in a very short time

Table 1. Frequency of different EPTB specimens received in Laboratory

Sr no.	Nature of specimen	No of samples received	No of Samples MTB detected	% age of detection
1	Gastric aspirate	109	9	8.25%
2	Pleural fluid	80	6	7.5%
3	CSF	47	6	12.7%
4	Pus	24	6	25%
5	Ascitic fluid	18	0	0%
6	Fluid	16	2	12.5%
7	Urine	15	3	20%
8	Drain	5	0	0
9	Stool	5	0	0
10	Synovial fluid	5	0	0
11	Biopsy	5	0	0
12	Abscess	4	1	25%
13	Pericardial fluid	2	0	0
14	Skin lesion	2	1	50%
15	FNAC	2	1	50%
	Total	339	35	10.32%

Gene Xpert results showed that 89.7% of the sample give results with not detected whereas remaining 10.3% of the study samples showed positive results for the presence of MTB (Table 2).

Methodology

The present study was a descriptive study that was done to observe yield of Xpert MTB/RIF assay in the samples of Extra-pulmonary TB for the period of six months i.e June 2018 to June 2019 at Lahore General Hospital. The specimens of the patients, who are clinically suspected as having TB other than lungs e.g Pleural effusions, lymph node biopsies, pericardial fluids, ascetic fluids, Cerebrospinal Fluids, urine, joints fluid, bone marrow aspirates, gastric aspirates, stool and skin scrapings were included in the present study. Specimens of pulmonary tuberculosis like Sputum, Broncho-alveolar lavage and blood were excluded from the study.

The sample reagent provided with cartridge is added in to the sample with 2: 1 and this mixture is incubated for 15 min at room temperature. The incubation

reduced the viability of MTB at 106 fold in the sample and thus biohazard of MTB. The cartridge is then filled with that mixture and loaded into the GeneXpert machine. The rest of the process of machine is fully automated which included automatically sample filtration and washing. Ultrasonic lysis of bacteria occurred that release its DNA. These DNA molecules are mixed with dry PCR reagent. Hemi-nested amplification and detection is carried out and in the end, printable DNA result in 1 hour and 45 min is displayed on the screen.⁹

For the study purpose strong inclusive and exclusive criteria were followed. Inclusion criteria was that only samples of EP-TB patients with sufficient amount was processed, whereas samples of pulmonary tuberculosis e.g. sputum and broncho-alveolar lavage and samples with insufficient amount of extra-pulmonary TB patients were excluded.

Table 2: Yield of Gene Xpert in EPTB specimens

Gene Xpert results	Frequency	Percentage %
Not Detected		
	304	89.7%
Detected		
Very low	15	4.4 %
Low	15	4.4 %
Medium	4	1.2 %
High	1	0.3 %
Total	339	100 %

Results

Total 339 samples of extra-pulmonary TB (EP-TB) were collected and processed for the study purpose. Among study cases, minimum age was 0.08 years and maximum age 85 years with mean 24.5 years with standard deviation 21.23. Among study cases 183 (53.98%) samples were received from males and 156 (46.02) samples from female patients.

Most of the samples (35.0%) were received form Pulmonology Unit followed by the samples (32.0%) received from Peads Unit (Figure 1).

Discussion

Approximately 15–20% of TB cases are estimated that they are from extra pulmonary sites and still existing data is missing from high burden countries.¹⁰ Diagnosing EPTB is always a challenge because the

clinical samples collected for its diagnosis are quite inaccessible and paucibacillary in nature, which decrease the diagnostic sensitivity of the samples. As the conventional microscopy for AFB in smears also has low sensitivity, range of 0% -40% and its negative results cannot eliminate the occurrence of TB. Similarly, yield of culture for mycobacterium varies and is gold standard for diagnosis yet it takes 2-8 weeks, which is very slow to start treatment options.¹¹

Detection of Mycobacterium by MTB/RIF assay Gene-Xpert is a hasty gene based molecular assay, which allow the diagnosis of Tb along with Rifampicin resistance in a short time of less than 2 hours with least technical expertise with extra benefit of direct sample assay of both pulmonary and extra pulmonary TB.¹²

In our study total 339 number of EPTB samples were received. Out of these samples, only 35 specimens

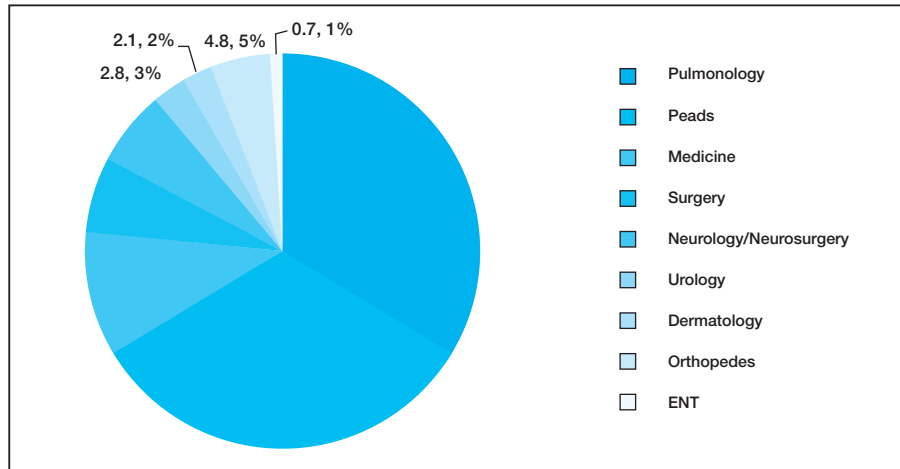


Figure 1: Frequency of wards from where EPTB samples were received.

showed detection of MTB, this was very low positive yield of test. In our study majority of extra pulmonary samples received were gastric aspirate, 109 samples of gastric aspirate/lavage were received out of which only 8.2% were MTB detected. Pang et al in 2014 concluded that Xpert MTB/RIF assay is an excellent tool for diagnosing smear-negative Gastric aspirates in childhood.¹³ The second most common samples received were pleural fluid, 7.5% were detected positive. Mishra et al in 2017 concluded low sensitivity of Xpert MTB/RIF assay in pleural fluid among high prevalence settings.¹⁴

Forty Seven (47), samples of CSF were received out of which 14.8% were detected for MTB, which was highest positive yield among our samples. Metcalf et al in 2018 also suggested in their study to support use of Gene Xpert as first line analytical tool for the patients of Tuberculosis meningitis. They also concluded that Gene Xpert also provides faster analysis as compared to culture and it provides rapid detection of rifampicin-resistance in the samples, which will help in the treatment of these critical patients of meningitis.¹⁵ In our study, we also diagnosed Mycobacterium with the help of Gene Xpert from pus, skin lesion biopsies and urine. Shakeel et al in 2018 conducted a study on pus specimens and concluded MTB RIF assay as a valid test for detection of AFB in pus specimens.⁶

Moreover, we did not get positive results from any specimens of stool, pericardial fluids, synovial fluid, ascetic fluid and abscesses. Rakotoarivelo et al 2018 documented that though nucleic acid amplification Gene Xpert facilitated diagnosis of TB in extra-pulmonary samples yet its sensitivity is poor in some of EPTB samples and these samples need more attention as compared to pulmonary samples.¹⁶ Contradictory to our study, a study conducted in

Pakistan KPK, in which they concluded MTB RIF assay as sensitive test for detecting EPTB in ascetic fluid and tissue specimens, supporting WHO guidelines regarding use of GeneXpert for extra pulmonary detection of TB in EPTB specimens.⁵

Conclusion:

Yield of MTB RIF assay was low in our study so we can concluded that though GeneXpert MTB/RIF assay is a very sensitive nucleic acid amplification tests and it makes diagnosis of MTB more reliable and its timely reporting yet we observed in our study that clinically this test is over-prescribed.

Recommendation:

We recommended that Xpert MTB/RIF assay should be ordered more specifically as this test is very expensive and sponsored by Global funding so we should not waste our resources for this sensitive but costly test.

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