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Role of automated GenexpertMTb/Rif system for rapid detection of Mycobacterium tuberculosis and Rifampicin resistance in extrapulmonary tuberculosis: A prospective study

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ABSTRACT

Introduction: Extrapulmonary Tuberculosis is infection of mycobacterium tuberculosis involving organ other than lung. It account for 15-20% of all TB cases. Various combined approach used for diagnosis of Extrapulmonary TB. GeneXpert MTB/RIF System is cartridge based nucleic acid amplification test to detect tuberculosis and rifampicin resistance in two hours.
Materials and Methods: This hospital based prospective study was carried out for period of 6 months with objective:-1) To evaluate the efficacy of GeneXpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis (EPTB) by comparing it with composite reference standard which include smear microscopy, clinical findings at time of presentation and three months follow up and radiological investigations.2) To look for Rifampicin Resistance by GeneXpert.
Results: Out of total of 168 enrolled patients 135 patients met the criteria of composite reference standard (CRS). Among total 135 CRS+ve EPTB Cases, 23 were paediatric (below 12 years of age) and 112 were Adults. Smear Microscopy was positive in 18 patients while GeneXpert gave positive results in 38 patients (8 paediatric and 30 adult patients). Rifampicin resistance was detected in 4 patients. Pleural effusion is the most commonly obtained sample followed by CSF, lymphnode, ascitic, urine, other fluid.
Conclusions: Sensitivity of GeneXpert is more as compared to smear microscopy. GeneXpert showed better sensitivity in CSF, tissue, lymphnode, pleural and urine. So early microbiological diagnosis and Rifampicin drug sensitivity in Extrapulmonary sample lead to early treatment initiation of Tuberculosis and decrease morbidity and mortality due to tuberculosis. RNTCP has also recommended use of GeneXpert in suspected TB in Extrapulmonary sites.

1. Introduction

Tuberculosis is infectious disease caused by Mycobacterium tuberculosis. India is high burden TB country. The estimated TB incidence in India is 27 lakh.[1] Tuberculosis typically affects the lungs (pulmonary TB) but can affect other sites as well (Extrapulmonary TB). Extra-pulmonary tuberculosis is defined as any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. abdomen, genitourinary tract, joints and bones, lymph nodes, meninges, pleura, skin.[2] Worldwide, Extrapulmonary Tuberculosis (EPTB) accounts for15-20% of all TB cases, and even higher percentages in HIV-infected individuals and children. In HIV-positive people, it accounts for 40-50 per cent of new TB cases.[3] Cervical Lymph node TB (LNTB) is the commonest form of EPTB.[4] Of the extrapulmonary forms of TB, tuberculous meningitis (TBM) is the most severe.

High degree of clinical suspicion is necessary for diagnosis of extrapulmonary TB due to its clinical presentation and

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simulating other neoplastic and inflammatory condition.[5]

Effort should be made to establish microbiological confirmation in case of presumptive EPTB. For diagnosis of EPTB several combined approach used like smear microscopy, culture by phenotypic or genotypic method, histopathological examination, Mantoux test, Interferon gamma release assay (IGRA), etc.

Smear microscopy has limited benefit in extrapulmonary sample due to its paucibacillary nature.[6] It cannot differentiate mycobacterial infection from non tuberculous mycobacterial infection. Therefor culture or molecular technique is required for diagnosis. But culture takes 6-8 weeks for diagnosis. It requires biosafety laboratory set up, trained and skilled staff for media preparation, processing and diagnosis. So culture diagnosis is time consuming.[6] Histological examination has limitations as it cannot differentiate between TB and non-TB infections caused by other related diseases e.g. sarcoidosis or NTM infections.

The Mantoux test gives rise delayed hypersensitivity reaction in form of induration. False positivity may be seen with previous bacillus Calmette-Guérin (BCG) immunization or exposure to atypical mycobacteria and false-negative results also occur due to poor technique of administration or reading the test and due to immunosupression.[7,8]

The typical histological findings of TB are granulomatous inflammation with central caseous necrosis. However, many diseases can cause similar changes, making it difficult to diagnose TB using histological findings alone.

GeneXpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA), a fully automated real time heminested PCR system detects TB and Rifampicin drug resistance in less than two hours.[9] It has minimal bio-safety requirements and training needs, and can be housed in non-conventional laboratories. GeneXpert MTB/RIF is unique because it is cartridge based nucleic acid amplification test in which sample processing and PCR amplification and detection are integrated into cartridge. These characteristics also make it a potentially attractive tool for extrapulmonary specimens. Presently under RNTCP, it is recommended for diagnosis of extra pulmonary TB also.

2. Materials and Methods

This hospital based prospective was carried out post ethical committee approval during period of February 2016 to September 2016 on patients of extra-pulmonary tuberculosis attending Department of Pulmonary Medicine at the SSG Hospital, Vadodara, Gujarat.

2.1. Inclusion criteria

1. Clinically suspected cases of Extrapulmonary Tuberculosis.
2. Adequate specimen material: 1 x 1 cm for biopsy, 3 ml for body fluids and 2.5 ml for CSF.

2.2. Exclusion criteria

1. Patient already diagnosed case of either pulmonary or extrapulmonary TB and already on antituberculous medication.
2. Inadequate specimen material.

After taking written and informed consent (from patient or guardian) about enrolment in the study and maintaining adequate privacy and confidentiality, all patients were subjected to standard interview. Complete medical history was obtained and complete general and systemic examination was carried out. Necessary investigations were performed according to the requirement of the patient to diagnose Extrapulmonary Tuberculosis. Suspected Extrapulmonary samples were collected under aseptic precautions and sent to RNTCP laboratory for Microscopy and Genexpert test.

2.3. Study objectives

1. To evaluate the efficacy of GeneXpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis by comparing it with composite reference standard which include smear microscopy, clinical findings at time of presentation and at 3 months follow-up and radiological investigations.
2. To look for Rifampicin Resistance by GeneXpert.

3. Results

A total of 168 patients were enrolled in the study. Of these, 135 patients met the criteria of composite reference standard (CRS) which included smear microscopy, clinical findings at time of presentation and 3 months follow up and radiological investigations for EPTB. Correlation between suspected TB cases and Composite Reference Standard is 80.35%. From the 135 CRS+ve EPTB cases, 93 patients were male and 42 were female. Median age of EPTB presentation in present study is 28. Male to female ratio in present study was 1.89:1. Among total 135 CRS+ve EPTB cases, 23 were paediatric (below 12 years of age) and 112 were adults.

Pleural effusion is the most commonly obtained sample (65%) followed by CSF (17.04%), lymphnode (6.66%), ascitic fluid (4.44%), urine (2.96%), other fluids (including-fluid from ovarian adnexa, pus from nephrostomy drain and joint fluid) (2.96%) and other tissue (which includes-endometrial biopsy) (0.7%) (Table 1, Figure 1).

Pleural effusion was the most common EPTB in both males and females. Lymph node TB was more common in females than male. Most common EPTB in and while in children, pleural effusion and TBME showed similar numbers in study group.

Table 1: Distribution of extrapulmonary sample and its microscopy and GeneXpert result

EPTB Samples	Total Suspected cases	CRS Positive Cases	Male	Female	Smear Microscopy		GeneXpert	
					+VE	-VE	+VE	-VE
Pleural Fluid	90	88	68	20	6	82	15	73
Ascitic Fluid	9	6	4	2	0	6	0	6
CSF	49	23	15	8	5	18	13	10
Lymph Node	9	9	2	2	5	4	7	2
Urine	5	4	3	6	0	4	1	3
Other Tissue*	2	1	0	1	1	0	1	0
Other Fluid#	4	4	1	3	1	3	1	3
Total	168	135	93	42	18	117	38	97

(# other fluid includes: Fluid from Ovarian adnexa, Pus from Nephrostomy drain and joint fluid. And *other tissue includes Endometrial biopsy)

Out of 135 CRS+ve EPTB cases, smear microscopy was positive in 18 patients while GeneXpert gave positive results in 38 patients (8 paediatric and 30 adult patients). So in this study smear microscopy had sensitivity 13.33, specificity 100%, Positive Predictive Value 100%, Negative Predictive Value 22%, diagnostic accuracy 33.35%. While GeneXpert had sensitivity 28.1%, specificity100%, Positive Predictive value 100%, Negative Predictive value 25.38%, Diagnostic Accuracy 42.26% (Tables 2 and 3).

We run the chi square test to compare the difference between sensitivity of AFB smear and Genexpert. Test showed p value of 0.0026, means difference between sensitivity of GeneXpert and AFB smear is statistically significant (Table 4).

In adults, smear AFB was positive in 17 patients out of 112 patients while GeneXpert was positive in 30 patients. In children, smear AFB was positive in 1 patient out of 23 patients while GeneXpert was positive in 8 patients. Sensitivity of smear was 15.78% in adult and 4.34%. In paediatric cases while sensitivity of GeneXpert was 26.78% in adults and 34.78% in paediatrics.

Comparison of sensitivity of GeneXpert v/s smear in the study, GeneXpert showed better sensitivity as compared to smear for pleural, ascitic, CSF, urine and lymphnode samples (Figure 2).

In the study, GeneXpert showed better results for CSF and urine samples in Paediatric population as compared to adults (70% for the CSF and 50% for urine in paediatric cases as compared to 46.2% for CSF and 0% for urine in adults), while for pleural fluid positive results are seen only in adult patients (19.2% in adults as compared to 0% in paediatric cases).

Out of total enrolled 168 patients, 8 were PLHA (People Living with HIV AIDS). Out of 8 PLHA patients, 6 patients met the CRS criteria. Amongst 6 CRS+ve PLHA patients, GeneXpert gave positive results in 5 patients while smear was positive in 3 patients. Sensitivity of GeneXpert in PLHA patients is 83.33% and Sensitivity of Smear in PLHA patients 50%.

Out of 38 GeneXpert positive samples Rifampicin resistance was detected in 4 patients (2 pleural fluid & 2 CSF samples). 1 CSF sample was from PLHA patient.

Table 2: Smear result

	CRS +ve	CRS –ve	Total
Smear +ve	18	0	18
Smear –ve	117	33	150
	135	33	168

(Sensitivity 13.33, Specificity 100%, Positive Predictive Value 100%, Negative Predictive Value 22%, Diagnostic Accuracy of Smear for AFB 33.35%)

Table 3: Genexpert result

	CRS +ve	CRS –ve	Total
GeneXpert +ve	38	0	38
GeneXpert –ve	97	33	130
	135	33	168

(Sensitivity 28.1%, Specificity100%, Positive Predictive value 100%, Negative Predictive value 25.38%, Diagnostic Accuracy 42.26%)

Table 4: Sensitivity ofGeneXpert v/s smear microscopy (Chi Square Test)

	GeneXpert	AFB smear
Positive	38	17
Negative	97	118
Total	135	135

(Sensitivity of Genexpert 28.1, Sensitivity of Smear Microscopy is 13.33,P value$<$0.0026

4. Discussion

Diagnosis of extrapulmonary-TB is challenging due to its paucibaciilary nature and inadequate sample volume.[9] Sensitivity of smear microscopy is less for EPTB samples as compared to sputum samples. Conventional culture method

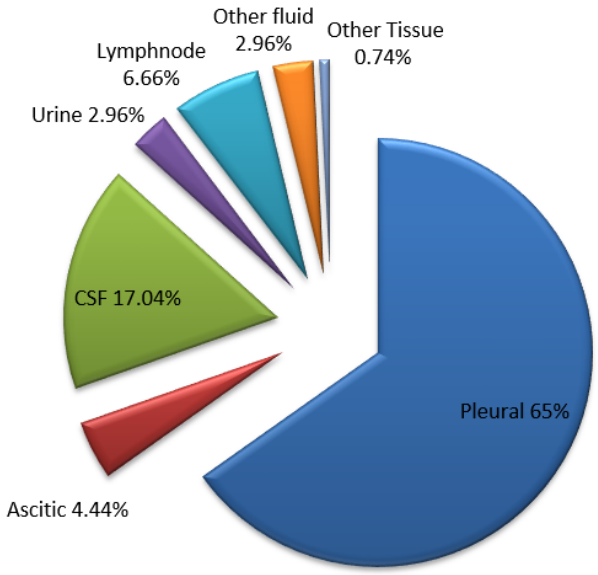

Fig. 1: EPTB Distribution among CRS+VE CASES

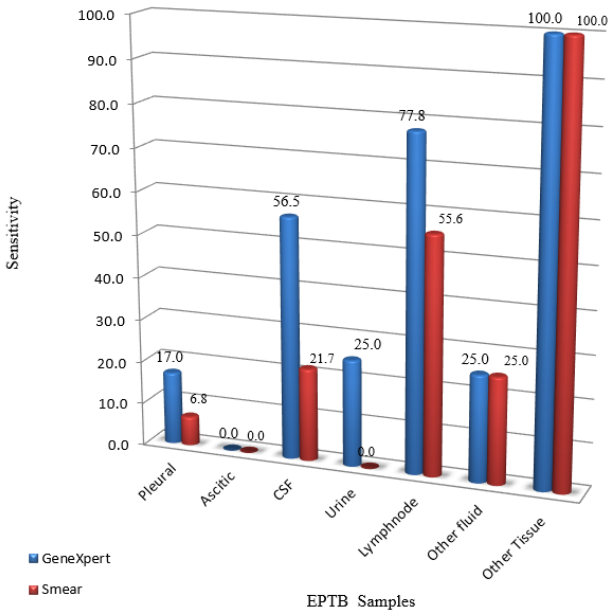

Fig. 2: Comparison of sensitivity of GeneXpert v/s smear for EPTB sample

for identification of mycobacteria and Drug sensitivity testing is complex and takes longer time. Genexpert is cartridge based nucleic acid amplification technique give result in two hour with Rifampicin drug sensitivity.

In present study Out of 135 CRS+ve EPTB cases, GeneXpert gave positive results in 38 patients while smear positivity seen in only 18 patients. So, GeneXpert is more sensitive than smear microscopy in present study.

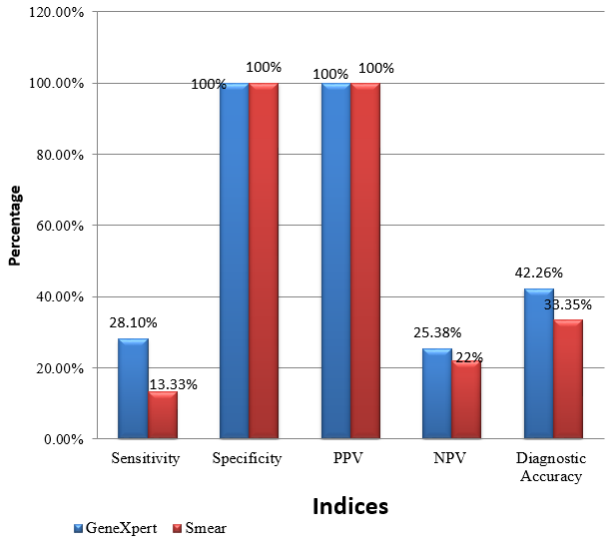

Fig. 3: Comparision of indices of GeneXpert and smear for AFB

Most common EPTB presentation in present study was pleural effusion. Similar observation is seen in study done by Avashia et al., and Hillemann et al.[10,11] Study by Hilemann et al., compared GeneXpert test assay with conventional liquid and solid culture method on EPTB sample showed combined sensitivity and specificity of the Xpert assay were calculated to be 77.3% and 98.2%, respectively.[11] Study done by Vadwai et al., showed the sensitivity of the Xpert assay was 81% with a specificity of 99.6%. The sensitivity was found to be high for the majority of specimen types (63 to 100%) except for cerebrospinal fluid, the sensitivity of which was 29%.[12]

In our study GeneXpert positivity was seen more in pleural fluid, CSF and lymphnode while study done by Avashia et al., showed maximum positivity in pus, pleural fluid and lymph node tissue.[10]

Sensitivity of GeneXpert for lymph node and tissue biopsy is more in the present study as compared to Vadwai et al., Hillemann et al., and Avashia et al., studies.

In the present study, Rifampicin resistance was detected in 4 patients out of 38 GeneXpert results. Among them, 2 samples were of CSF and 2 were of pleural fluid. So 4 patients were put on MDR TB regimen as per the RNTCP guidelines. So early detection of Rifampicine resistance by GeneXpert and early treatment initiation led to decrease morbidity and mortality related to drug resistance tuberculosis.

There were only 6 CRS+ve PLHA EPTB patients in the present study, out of which GeneXpert showed positive result in 5 patients with sensitivity of 83.33%. So early confirmed microbiological diagnosis and early diagnosis of Rifampicin resistance in HIV positive patients lead to early treatment initiation of tuberculosis and improved the quality of life.

So in present study sensitivity, negative predictive value & diagnostic accuracy of GeneXpert was better compared to smear microscopy, while specificity & positive predictive values of GeneXpert and smear were comparable (Figure 3).

5. Conclusions

The Xpert assay has brought about a major change in the speed, simplicity and accuracy of not only diagnosis of TB but also drug resistance to Rifampicin in TB, which is accepted as a surrogate for MDR-TB. This study showed better results in PLHA & paediatric patients in terms of rapid confirmed diagnosis of EPTB samples. In EPTB cases, GeneXpert showed better sensitivity in CSF, tissue, lymphnode, pleural fluid and urine.

RNTCP has also recommended use of GeneXpert in suspected TB in extrapulmonary site if adequate sample available for process. So early microbiological diagnosis and Rifampicin drug sensitivity in extrapulmonary sample lead to early treatment initiation of Tuberculosis and decrease morbidity and mortality due to tuberculosis.

6. Source of Funding

None.

7. Conflicts of Interest

None declared.

8. Acknowledgment

Nil.

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