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Role of gene Xpert MTB/RIF in diagnosis of Extrapulmonary Tuberculosis at Government Medical College, Amritsar

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Abstract

Background- Tuberculosis (TB) remains one of the most significant causes of death from an infectious agent**.** Approximately 15 to 20% of tuberculosis cases in India are estimated to have extrapulmonary tuberculosis (EPTB), and due to its paucibacillary nature and lack of diagnostic test, they often remain undiagnosed or misdiagnosed. The World Health Organization has recently endorsed the Gene Xpert MTB/RIF assay for rapid detection of EPTB.

Aims: Our study was done with an aim to know the role of Gene Xpert in the diagnosis of EPTB and detection of rifampin resistance.

Method- The study included 100 clinicoradiologically suspected patients of extra pulmonary tuberculosis, who came to outpatient department or admitted in wards. There biological specimen were collected and sent for Gene Xpert MTB/RIF assay and other relevant investigation.

Results: Out of 100 EPTB samples thus examined, 35 were positive for Mycobacterium Tuberculosis (MTB) by Gene Xpert i.e. average of 35%. Out of 35 cases diagnosed as EPTB, 3 were Rifampicin Resistant as detected by Gene Xpert.

Conclusion: Gene Xpert MTB/RIF assay is rapid method for diagnosis of extra pulmonary tuberculosis as compared to conventional methods. Because of its simplicity, rapidity and sensitivity, this seems to be a very important test for diagnosis of EPTB from clinicoradiologically suspected samples.

Keywords: EPTB; Gene Xpert MTB/RF; Rifampcin resistance; Mycobacterium Tuberculosis; paucibacillary.

Introduction

Tuberculosis (TB) is a global health concern. South East Asia carries about 40% of the global TB burden. India is highest TB burden country in the world and accounts for one fourth of the global TB burden cases. In 2015, an estimated 28 lakh cases occurred and 4.8 lakh people died due to TB.[1]

Tuberculosis can involve any organ system in the body. While pulmonary tuberculosis is the most common presentation, extra pulmonary tuberculosis (EPTB) is also an important clinical problem.[2-4] The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung. However, when an extra pulmonary focus evident in a patient with pulmonary tuberculosis, such patients have been categorized under pulmonary tuberculosis as per the guidelines of the World Health Organization (WHO).[5]

Extra pulmonary Tuberculosis (EPTB) accounts for about 15 to 20% of all cases of Tuberculosis in India.[6] It can affect any organ of the body, most common type is lymph node TB and then pleural effusion, tubercular meningitis, abdominal TB, bone TB are other form of EPTB. The percentage may be higher in children and in HIV infected individuals.[7] In HIV positive patients, EPTB accounts for more than 50 per cent of all cases of TB.[8]

Extra pulmonary infections with members of the Mycobacterium tuberculosis complex (MTBC) have high morbidity and mortality because of lack of good diagnostic methods. Diagnosis is often difficult to establish due to low number of bacteria and collection of extra pulmonary samples is not easy. A definitive diagnosis of mycobacterium infection depends on detection of the Mycobacterium Tuberculosis in extra pulmonary samples.[9]

In December 2010 WHO endorsed the Gene Xpert MTB/RIF technology and released a recommendation and guidance for countries to incorporate the new test into their programmes. In 2013, the World Health Organization (WHO) endorsed the use of Gene Xpert MTB/RIF assay, A cartridge based nucleic acid amplification test (CBNAAT) for EPTB.[10] In March 2014, the 3rd edition of the updated International Standards for TB Care (ISTC) and the first edition of the Standards for TB Care in India (STCI) were released and both included new recommendations for the diagnoses of EPTB.[11]

Materials and Methods

This study was conducted in the department of Chest and Tuberculosis, Government Medical College, Amritsar. The study included 100 clinicoradiologically and histopathologically suspected patients of extra pulmonary tuberculosis, who came to outpatient department or admitted in wards. This study was conducted after approval from the Institutional Ethics Committee and informed consent of each patient.

Inclusion criteria:

1. Patients having clinical feature of extra pulmonary tuberculosis.
2. Patients age more than 12 year of age.
3. Patients giving consent for the study.

Exclusion criteria:

1. Patients of sputum positive pulmonary tuberculosis.
2. Patients on antitubercular treatment for more than 1 month.
3. Patients age less than 12 year of age.
4. Patients not giving consent for the study.

The Gene Xpert MTB/RIF assay detects DNA sequences specific for Mycobacterium tuberculosis and Rifampicin resistance by polymerase chain reaction (PCR). CBNAAT is cartridge-based nucleic acid amplification test having fully integrated and automated amplification and detection using real time polymerase chain reaction, providing result within 2 hours, It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target rpoB gene of MTB, no cross-reaction have been observed with other bacterial species tested, thereby excluding non-tubercular mycobacterium (NTM). It is based on the Cepheid Gene Xpert

MTB/RIF system, a platform for rapid and simple-to-use. The Gene Xpert MTB/RIF purifies and concentrates the Mycobacterium tuberculosis bacilli from extra pulmonary samples, isolates genomic material and amplifies the genomic DNA by PCR. Results are obtained within 2 hours from extra pulmonary samples, with minimal biohazards. Further minimal technical training is required to operate the instrument. These features make it an important tool for extra pulmonary samples.[12]

All specimens were collected & transported whenever possible at 2 to 8°c in pre-sterilized falcon tubes and if sufficient volume of specimen is available, concentration methods used to increase yield by centrifuge technique. In lymph node and other tissue, 2ml of sterile phosphate buffer solution (PBS) is added, from this 0.7 ml of homogenised tissue specimen is collected and mixed with double volume of Gene Xpert sample reagent (1.4ml).The processed sample is loaded into Gene Xpert MTB /RIF cartridge. The automated generated result was available in abouts 100 minutes.

Results

All the samples of pleural fluid, ascitic fluid and CSF had exudative nature in biochemistry examination, lymphocytes predominance cell by cytological examination and ADA value was more than 40 IU for pleural fluid, more than 30 IU for ascitic fluid and more than 10 IU for CSF fluid. Pus aspirated from various sites of the body was examined for ZN smear microscopy for AFB and Gene Xpert assay. This study was done to determine the role of Gene Xpert in diagnosis of clinicoradiologically and histopathologically suspected cases of extra pulmonary tuberculosis. The observations and results of the studied patients were recorded and tabulated as follows:

Table 1 Distribution of samples according to various samples examined

Samples	Number of cases	Percent
Pus	38	38%
Pleural Fluid	32	32%
CSF	18	18%
Ascitic Fluid	12	12%
Total	100	100

Graph 1: Showing distribution of samples according to various samples examined

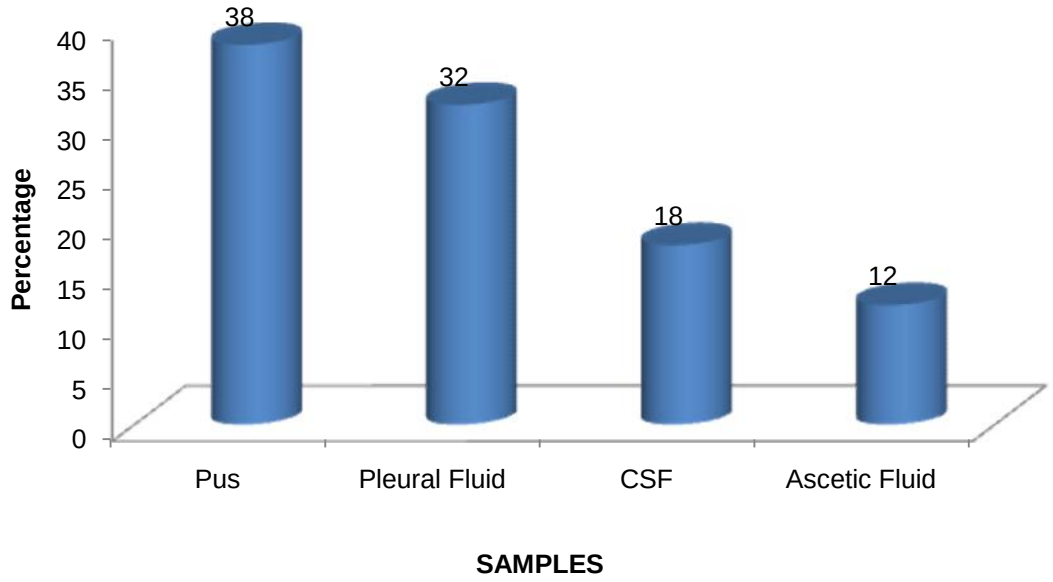


Table 2 Distribution of samples according to examination of ZN smear for AFB by microscopy

Samples	ZN smear for AFB by microscopy		Total
	Positive	Negative	
Pus	7	31	38
Pleural Fluid	-	32	32
Ascitic Fluid	-	12	12
CSF	-	18	18

Graph 2: Showing distribution of samples according to ZN smear for AFB by microscopy

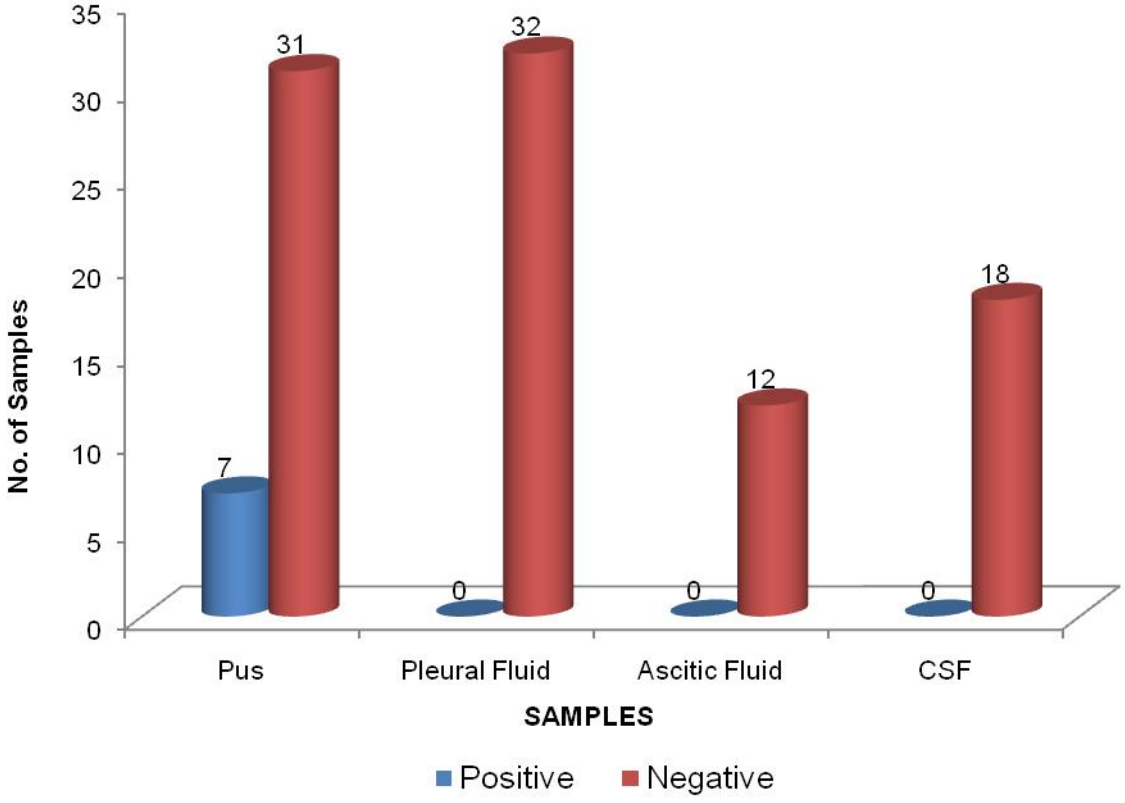


Table 3 Distribution of samples analyzed by gene Xpert MTB/RIF Assays

Result of gene Xpert MTB/RIF Assay					
Type of Samples	No. of samples	Positive	Percent	Negative	Percent
Pus	38	21	55.2%	17	44.1%
Pleural Fluid	32	7	21.8%	25	78.2%
Ascitic Fluid	12	1	8.3%	11	91.7%
CSF	18	6	33.3%	12	66.7%
Total	100	35	35.0%	65	65.0%

Graph 3: Showing distribution of samples analyzed by gene Xpert MTB/RIF Assay

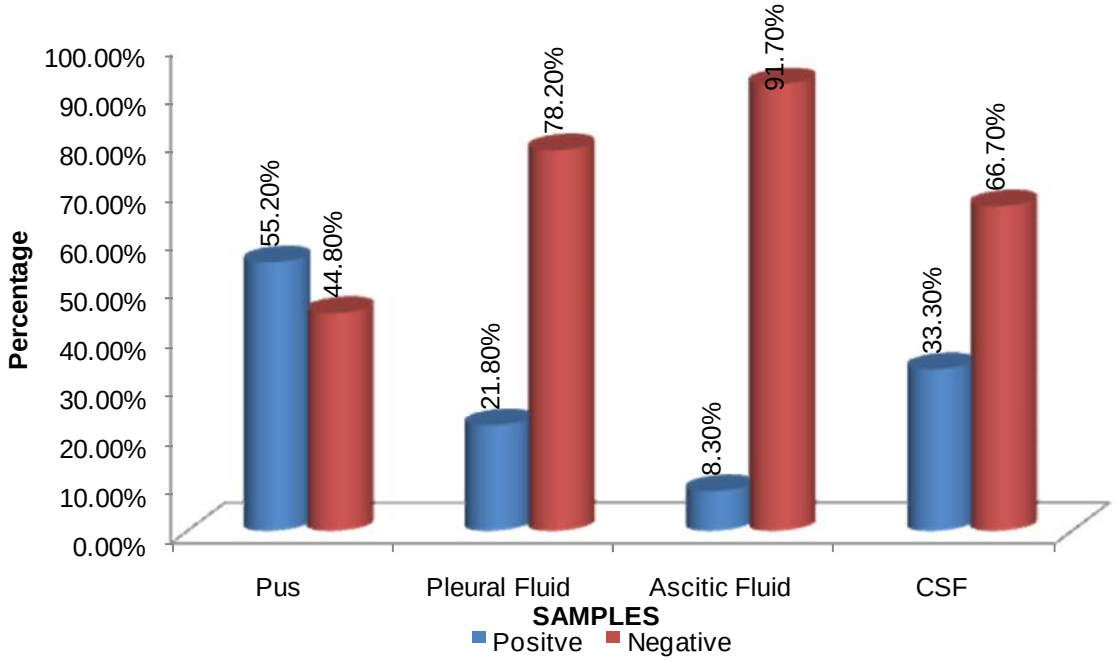
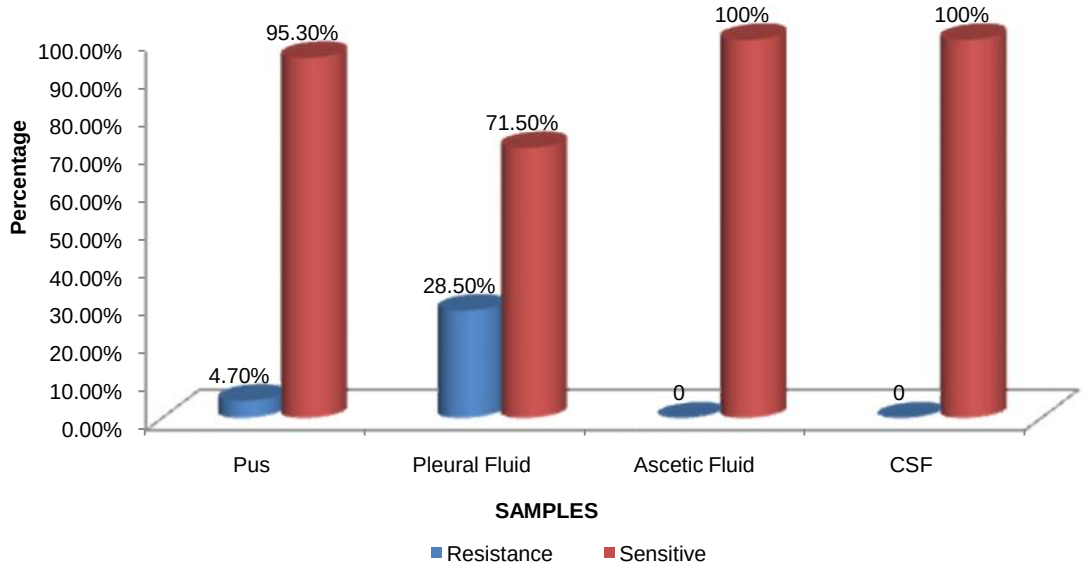


Table 4 Distribution of patients according to Rifampicin sensitivity analyzed by gene XPERT MTB/RIF Assays

Rifampicin				
Type of Samples	Resistance	Percent	Sensitive	Percent
Pus	1	4.7%	20	95.3%
Pleural Fluid	2	28.5%	5	71.5%
Ascitic Fluid	-	-	1	100%
CSF	-	-	6	100%
Total	3	8.5%	32	91.5%

Graph 4: Showing distribution of patients according to Rifampicin sensitivity analyzed by gene Xpert MTB/RIF assay



Discussion

Diagnosing EPTB remains challenging because of various reasons: symptoms varying depending on the organ involved, clinicians having low level of suspicion because of varied presentations, clinical samples obtained from relatively inaccessible sites being paucibacillary; all these factors decreasing the sensitivity of diagnostic tests. Since the conventional smear microscopy has a low sensitivity with a range of 0%–40%, negative results cannot exclude the presence of TB.[13] The reported yields of mycobacterium culture vary from 30% to 80%, but it usually takes 2–8 weeks to receive the results, which is too slow to help treatment decisions.[13] All these factors lead to a delay in diagnosis. Accurate and rapid laboratory investigations have therefore gained importance. Gene Xpert is one such useful and rapid laboratory investigation for diagnosis of TB and detection of Rifampicin Resistance.

In our study, we had 38 pus samples aspirated from various sites of body (lymph node, empyema, cold abscess, psoas abscess), 32 pleural fluid samples, 18 CSF samples and 12 ascitic fluid samples.

In present study, out of total 38 pus samples 7 (18%) were AFB positive by smear microscopy and all the samples of pleural fluid, ascitc fluid and CSF were negative for ZN smear microscopy for AFB. Conventional smear microscopy for EPTB samples has a low sensitivity with a range of 0%–40%.[13] Correlation between pus for AFB by ZN smear microscopy and pus for Gene Xpert was statistically not significant ($p>0.05$).

In this study, the overall diagnostic yield of Gene Xpert MTB/RIF in various extra pulmonary samples in order of frequency is as follows: Pus 21 (55.2%) out of 38 samples, CSF 6 (33.3%) out of 18 samples, pleural fluid 7 (21.8%) out of 32 samples, and lastly ascitic fluid 1 (8.3%) out of 12 samples. Thus pus has the maximum diagnostic yield. Out of 100 samples thus examined, 35 were positive for Mycobacterium Tuberculosis (MTB) by Gene Xpert i.e. average of 35%. Out of 35 cases diagnosed as EPTB, 3 were Rifampicin Resistant as detected by Gene Xpert. Among these 3 Rifampicin Resistant cases, 2 were of pleural fluid, 1 was of pus. All CSF and ascitic fluid samples were Rifampicin sensitive.

These findings of our study were similar to the study conducted by Srwar A et al[15] in which the yield of Gene Xpert was 31 out of 60 (51.7%) pus samples, 3 out of 19 (15.8%) pleural fluid samples, 1 out of 16 (6.3%) ascitic fluid samples and 2 out of 5 (40.0%) cerebrospinal fluid samples.

Similar study was also done by Keny SJ et al[16] at Goa medical college in which Out of 212 samples 51 were positive for Mycobacteria Tuberculosis (MTB) by Gene Xpert i.e. average of 24%. Out of 51 cases which were positive by Gene Xpert, 38 were of lymph node TB i.e. 64%, 6 of TB pleural effusion i.e. 7.3%, 2 of urogenital TB i.e. 16.6%, 2 of abdominal TB i.e.16.6%, 1 of CNS TB i.e. 3.4%, 2 of TB involving the skeletal system i.e. 25%. Out of 51 cases diagnosed as EPTB, 5 were Rifampicin Resistant as detected by Gene Xpert. Among these 5 Rifampicin Resistant cases, 3 were of lymph node TB, 1 Was of scrotal TB and 1 was of abdominal TB. The finding of this study was in concordance with our study.

An overall sensitivity of 83.1% and a pooled specificity of 98.7% for diagnosis of EPTB was recently published in a meta-analysis by Denkinger et al.[7] Raquel Moure et al[17] in 2012 conducted a study; in which 58.3% were positive with the Xpert MTB/RIF assay for Mycobacterium tuberculosis. In a similar study by Vadwai V et al[18] in 2011, the sensitivity of the Xpert assay was 81% (228/283 specimens), 64% for smear-negative cases and 96% for smear-positive cases, with a specificity of 99.6%.

The higher detection rate in above mentioned studies was due to the fact that they included diagnosed cases of TB (culture positive, smear positive) while our study was performed on suspected cases of EPTB.

Gene Xpert thus proved to be a useful investigation in diagnosis of EPTB mainly tubercular pus and CSF in our study. Also, Rifampicin resistance was detected rapidly in 3

EPTB cases. So far, there was not much information available about drug resistance in EPTB precisely because of same reasons i.e. difficulty in obtaining adequate samples for investigation and limited availability of drug sensitivity testing facility. However, with recent advances in diagnostic investigations of TB one of which is Gene Xpert, the diagnosis of EPTB as well as Rifampicin resistant EPTB is going to be easier and faster. However clinicians will have to keep in mind EPTB while investigating patients with clinical presentation suspicious of EPTB and make use of this very important and valuable tool as it is made available through Revised National TB Control Programme (RNTCP) all over the country. Also according to the RNTCP guidelines, one indication of use of Gene Xpert is for diagnosis of EPTB. This study justifies its use in some types of EPTB especially tubercular pus and CSF where as recommendation of not using it in tubercular pleural effusion and ascitic fluid as per INDEX TB guidelines 2017[19] is also justified in this study.

Conclusion

Gene Xpert MTB/RIF assay is efficient and reliable technique for the rapid diagnosis of extra pulmonary TB, especially in suspected cases. Its simplicity, speed and automation, make this technique a very attractive tool for diagnosis of Mycobacterium tuberculosis from extra pulmonary samples of TB suspects. Our findings suggest that Gene Xpert may have a role in EPTB diagnosis in addition to PTB, particularly in low-income/high-burden settings, where facilities for mycobacterial culture are limited.

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Conflict of interest: None declared

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